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## SEEDLING DISEASES OF CONIFERS

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### INTRODUCTION

Because of the high cost of seed and the slow growth and delicate character of seedlings during the first few weeks after germination losses caused by the disease known as damping-off have been an important factor in the propagation of conifers. The fact that a number of damping-off parasites are able to cause practically identical symptoms, and the further fact that certain physical factors sometimes cause injury resembling damping-off, has made a study desirable, both of damping-off and of the other diseases which may attack seedlings of the same age. The present paper will consider all the diseases which the writers have found attacking seedlings up to the age of approximately two months.

### DAMPING-OFF

Damping-off is the most serious of the diseases attacking coniferous nursery stock in most regions. There is considerable literature on the disease both in connection with conifers and with truck crops. The most important work in relation to conifers is summarized by Spaulding (*25*).[2] Both etiology and control are, nevertheless, seriously in need of further investigation, because of the complications arising from the multiplicity of hosts and parasites involved and from the soil-inhabiting tendency of several of the parasites. A summary of the available data on control has been recently published (*16*).

#### TYPES OF DAMPING-OFF IN NURSERIES

The old conception of damping-off seems to have been the death of seedlings as the result of the attack of a parasitic fungus at the soil surface, causing a local constriction of the stem at that point, followed by the fall and wilting of the seedling. It appears that several parasites

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[2] Reference is made by number (italic) to "Literature cited," pp. 556–558.

may attack seedlings in this manner, but that they also very frequently attack the seedlings in other ways, while the cases which most closely agree with the older descriptions of damping-off are often not caused by parasites, and may occur under hot, dry conditions. The term "damping-off" as here used will include all cases of the death and early decay of seedlings less than 2 months old, resulting primarily from fungus invasion. Consideration of the disease as thus limited will therefore involve the discussion both of the work of several different parasites and of several symptomatic types. Many of these types intergrade to such an extent that there is no possibility of giving them separate treatment as distinct diseases. The different types recognized are described in the following paragraphs.

### NORMAL DAMPING-OFF

Normal damping-off is caused by *Pythium debaryanum*, *Fusarium moniliforme*, *F. ventricosum*, *Corticium vagum* (the common American Rhizoctonia), and several other fungi. The still succulent seedlings are invaded by the parasites at any point on the root or lower part of the stem, ordinarily a short distance below the ground surface. The parasites spread rapidly, especially through the root tissues, and the seedlings fall over. The fall of the seedlings is not usually due to stoppage of water supply and consequent wilting; it most commonly occurs when the tissues of the hypocotyl just above the soil surface become involved in the decay, and while the rest of the stem is still green and turgid. This type of disease is shown in Plate B, figure 1. It may be fairly well controlled by soil disinfection.

### GERMINATION LOSS

This type of damping-off seems in most cases to be caused by species of Pythium and Corticium rather than by Fusarium. The radicles are killed soon after emerging from the seed coats and before the seedlings appear above the ground. Two-thirds of the seedlings are sometimes destroyed in this way in nursery beds. There is no essential difference between this type and the normal type, except that this type occurs earlier in the life of the seedling and is ordinarily not detected. A poor initial stand of seedlings due to this type of damping-off is commonly attributed by the nurseryman not to damping-off but to poor germinative capacity of the seed. This early type of damping-off, like the normal type, is invariably fatal. It can be much more completely prevented by soil disinfection than can the more familiar normal type.

It is often stated by practical men that seedlings from seed with low germination percentage are especially liable to attack by damping-off fungi. This is very likely true within certain limits. However, in a great many cases in which apparently poor germination has been followed by especially heavy damping-off losses, the sequence is to be

attributed not at all to poor seed, but rather to abundant early infection of beds with species of Pythium or Corticium, causing both the apparently poor germination and the high damping-off loss.

### LATE DAMPING-OFF

The term "late damping-off" is used for the damping-off as a result of root infections of seedlings several weeks old whose stems have developed strong supporting tissues.

The parasites which cause normal damping-off are also probably responsible for most cases of this type. Like germination loss, it differs from the normal type only by the age of the seedlings concerned. However, the symptoms of late damping-off are very different from those of the normal type; the seedlings remain erect, dry up and turn brown, and in some cases even shed their leaves before the stem finally falls over (Pl. B, fig. 6). This type is very likely to be confused with drouth injury.

The death of seedlings due to root killing by damping-off parasites in rare cases continues throughout the season, and probably even into the second year in cases where development of the host species has been especially slow. This appeared to be the case with Engelmann spruce (*Picea engelmanni*) at a mountain nursery in New Mexico. All such death, up to the rather arbitrary age of two months, is classed by the writers as damping-off, while similar death of still older stock is classed as rootrot. This time limit is justified only by convenience. The damping-off parasites do practically all of their damage at most nurseries before the seedlings are 2 months old. Their work up to that time is fairly easy to detect and distinguish from disease due to other causes, because of the localization of most of the affected plants on the margins of the old damping-off patches of normal type. Furthermore, the soil treatments which control the normal type of damping-off are also useful, at some places at least, in lessening damage from the late type of disease. It is therefore easiest to consider all the trouble during the first two months as damping-off, without trying to mark any of the intermediate stages as distinct diseases.

The death of stock over 2 months old, on the other hand, will require both different investigative methods and, very likely, different types of control measures. Although sometimes caused by damping-off organisms, there seems to be no practical advantage in considering it at the same time as damping-off.

The impression prevalent in some places that damping-off organisms always infect at the soil surface is shown to be especially incorrect in the case of this late type of damping-off. In one case the root of a young seedling of western yellow pine (*Pinus ponderosa*), growing rapidly in sandy soil at the outer margin of a damping-off patch, was found to have been attacked at a point 11 inches below the soil surface. Not all

the seedlings affected by late damping-off are killed by the parasites. Many of them, as in the case of the "root-sick" sugar-beet seedlings described by Edson (7), have only the youngest portions of their roots killed, and are able then to resist further attack, and so recover. Pine seedlings on the margins of damped-off areas can be found at the age of 6 to 7 weeks, with more than half of their root systems decayed, but with the older parts of the root intact and with vigorous laterals starting from the terminal of the healthy portion. That such seedlings are capable of recovery was demonstrated by replanting a number of badly injured specimens of Corsican pine (*Pinus nigra poiretiana*)[1] and observing their growth during the subsequent weeks.

Pines affected with the late type of damping-off show no external evidence of disease until they are practically dead. Infected seedlings which recover do not, like the root-sick sugar beets described by Edson, exhibit noticeably arrested growth and a flabby appearance of the tops.

### DAMPING-OFF OF TOPS

A type of damping-off involving parts of the cotyledons or the upper stem, while the lower stem and root remain sound until after the death of the parts above, is fairly common under moist atmospheric conditions, as in greenhouses, although seldom as prevalent in open seed beds as the normal type of damping-off in which the root or the stem at the ground line is first attacked. Infections are believed in the main to be due to Fusarium. Under extremely moist conditions the mycelium may spread directly to the other seedlings whose cotyledons touch the diseased plant. Soil disinfection is, as would be expected, of relatively little value in preventing this disease of the tops. Avoidance of excessive atmospheric humidity appears especially important for control of this type.

A special case of damping-off of tops of seedlings is found in cases in which the tips of all the cotyledons are simultaneously killed. This at first suggests insufficient water supply as the cause, but examination of younger seedlings shows it to be parasitic. Infection occurs in such cases while all the tips are still inclosed in the persistent seed coat. This type of damping-off is thought to be caused most commonly by species of Fusarium, but it has been observed in pots whose soil had been inoculated with *Corticium vagum*, though absent in parallel control pots. It is also possible that *Pythium debaryanum* may be a cause of this type, as there is abundant opportunity for infection to occur before the tips of the cotyledons and the seed coat they carry are released from the soil. Soil disinfection has apparently decreased losses from this type of damping-off.

[1] The nomenclature used in this paper for foreign trees follows BAILEY, L. H. STANDARD CYCLOPEDIA OF HORTICULTURE. New York, 1916; and for native trees follows George B. SUDWORTH in various publications of the Forest Service, United States Department of Agriculture.

Both these types of damping-off of tops commonly kill the affected seedlings, but not always. Stem infections regularly cause death, but lesions originating in the cotyledons are often arrested before they progress far enough to kill the growing point in the center of the whorl of cotyledons.

### BLACKTOP

This type of damping-off known as black top is simply a special case of the preceding from which it is distinguished by the dark color of the tissues decayed. It is illustrated in Plate B, figures 2 and 3. Infection takes place at any point on the stem or cotyledons of very young seedlings. Both infection and the extension of the lesion seem to depend on special weather conditions. There is reason to believe that a species of Trichoderma is the direct cause. This type of damping-off is seldom sufficiently prevalent to be of importance. Soil disinfection with acid is apparently ineffective in preventing blacktop damping-off.

### DECAY OF DORMANT SEED

It is undoubtedly true that dormant seed are sometimes killed by microorganisms. Under ordinary seed-bed conditions, species of Pythium and Corticium probably kill some coniferous seed before the coats are split, although this has yet to be demonstrated. It is also considered probable that under certain conditions considerable quantities of seed are destroyed by ordinarily saprophytic molds, which possibly reverse the usual action of the damping-off parasites by attacking the cotyledons before the embryonic radicle is invaded. Seed of jack pine (*Pinus banksiana*) shipped moist in a tin container has been found seriously molded with a species of Penicillium fruiting vigorously on the outside of all the affected seeds. It is commonly stated that the seeds of many crop plants rot in the soil during prolonged wet weather. To what extent coniferous seed suffer from microorganisms before beginning to germinate can not be stated without further investigation. Although perhaps not strictly a damping-off problem, it is certainly one that can be considered to advantage in connection with work on damping-off proper.

### TYPES OF DAMPING-OFF IN FORESTS

In direct seeding by the seed-spot method in northern Idaho, the writers are advised by Mr. E. C. Rogers, of the Forest Service, that a considerable proportion of the seedlings die from what appears to be damping-off, and that cultural examinations have shown *Rhizoctonia* sp. in the majority of the cases.

In natural reproduction normal damping-off at least in most forests appears to be a much less important factor than in nurseries. Serious damping-off should be expected only where seedlings come up in groups, or where soil conditions especially favor parasites.

In cases of conifers which fail to reproduce well in forest soils containing much humus or covered with litter, the possibilities of parasitic germination loss or of the decay of seed before germination starts are to be considered. In stands of scrub pine (*Pinus virginiana*), loblolly pine (*P. taeda*), shortleaf pine (*P. echinata*), and pitch pine (*P. rigida*) in the vicinity of Washington, D. C., where recently germinated seedlings on bare mineral soils are numerous, search failed to show either seedlings or germinating seed in the litter at points where mineral soil was not exposed. As sufficient moisture was present in the upper layers of the litter, and seed of broad-leaved species was found germinating in fair quantity, suspicion is directed toward some biologic factor as the cause of the lack of conifers. It is suggested that the decay of dormant seed by the vigorous, ordinarily saprophytic mycelia present in leaf mold may prove an important cause of failure to secure natural reproduction of some conifers on humus soils. The entire matter of the importance of the different types of damping-off in limiting reproduction in coniferous forests needs investigation.

## INOCULATION PROCEDURE

### STANDARD INOCULATION METHOD

Except where otherwise stated, all of the experiments whose results appear in this paper were conducted by the following methods:

Inoculum was added to the soil on which the seed was sown, instead of applying it directly to the seedlings themselves. The procedure was to sow the seed broadcast in pots of a mixture of sand and loam, in most cases containing some compost. The pots and contained soil were sterilized by steam pressure one to three days before seed sowing. The inoculum was laid on the soil at the time seed was sown and both inoculum and seed covered to the depth of about ¼ inch with additional soil or sand, also steamed. Three-inch pots were most commonly used, and all pots received equal quantities of seed, determined in some experiments by count, in some by weight, and in some by volume, or quantities proportional to their areas in the few cases in which pots of different sizes were used in the same experiment. The number of seeds used was in most cases somewhat greater, and in a few cases much greater, than would have been used on an equal area of seed bed in ordinary nursery operations. Partial seed disinfection by sulphuric acid was practiced in a few cases, but not for the most part, as there are no indications that the seed carry parasites to any considerable extent. The inoculum used was the surface layer of an apparently pure culture of the test fungus on prune or corn-meal agar, occasionally on steamed rice or corn-meal mush, and therefore included both nutrient substratum, mycelium, and in most cases spores, except in the case of species of Corticium and Botrytis, which were not observed to form spores on any

of the artificial media used. In typical experiments the inoculum fragments were distributed among the seed over a sector of the pot approximately one-fourth the area of the surface sown. In special cases the inoculum was limited to one or two fragments, while in other experiments fragments were distributed over the entire surface of the soil. In a few of the earlier experiments pots were covered, except during the seed-sowing process, until germination. Stands whose legs were set in pans of water were used in most of the experiments to exclude slugs and ants. In the majority of the inoculations the control pots were given sterile agar from the same lot as that serving as substratum for the cultures placed in the inoculated pots. Results in such experiments were not noticeably different from those in which the controls received no agar. In the later experiments all watering was with heated water, resulting in a decrease in the amount of contamination as indicated by a lesser damping-off occurring in the control pots.

Reisolations, like the original isolations, were made by planting recently affected seedlings in solidified agar plates and transferring from the advancing margins of the resulting growth. Reisolations were made only in experiments in which the control pots had remained free from disease.

### DEFICIENCIES IN STANDARD METHOD

The above method of conducting inoculations, involving sterilized soil, heated water, heavy seeding, and heavy inoculation, is a convenient one. The first two of these features are necessary when it is desired to keep the controls entirely free from disease and reisolate the organisms used in the inoculation work. It is the method which has been used by most recent experimenters with root parasites. Attention should, however, be called to the fact that it is not a reliable index of what takes place in ordinary seed beds unless supplemented by experiments under more nearly natural conditions. It is well established that as a substratum for the growth of either higher plants or of fungi steamed soil is a very different thing from normal soil. The quantity of water-soluble matter, both organic and inorganic, is changed; the composition of the organic and inorganic matter is changed; and the effects of destruction of the original microflora and fauna, which can be hardly reestablished for several months in the original composition and balance, can scarcely fail to be reflected upon both hosts and parasites grown in the steamed soil. That the changes due to steaming are of more than theoretical importance is shown by the comparison of results of inoculations on steamed and unsteamed soil. Successful inoculation, at least with some of the parasites, seems much easier to secure in steamed than in normal soil. A further indication that heating soil is likely to abnormally favor damping-off parasites is seen in the heavy spontaneous losses occurring on soil subjected to temperatures of only 80° to 90° C. at some nurseries. In

field tests in the Middle West heated soil plots not specially protected against reinfection have in a number of cases suffered even more heavily from damping-off than plots not treated, despite the demonstrated killing of the parasites originally present in the soil.

Heavy inoculation with material consisting largely of substances like corn-meal mush, rice, and various nutrient agars, as has been customary, undoubtedly results also in soil conditions decidedly different from those which occur in nature. It is believed that the presence of abundant rich food material for the parasites decidedly increases the ability of some of them to attack the seedlings. Heavier seeding than that used in practical seed-bed work has been a part of the experimental procedure in most of the writers' work if not in that of others, and also appears to create conditions abnormally favorable to the parasites. It is felt that all of the work that has been done, including that presented in the following discussion, must be supplemented by further experiments on unsterilized soil, with unheated water, with a density of seed sowing corresponding to that used in regular seed-bed practice, and with inoculum consisting of spore suspensions where practicable, or with small quantities of mycelium in other cases, but in no case with any addition of a nutrient medium. Inoculation in outdoor seed beds, as well as in pots in greenhouses, will be desirable. Conclusive results in such experiments will not always be easy to get. Because of the inevitable damping-off in the controls of experiments so conducted, positive results will have to consist of a heavier damping-off loss than occurs in the controls, a thing which can be demonstrated only by averaging the results obtained from a large number of pots. Until such work is done the relative importance of the various parasites active under field conditions can only be guessed at, no matter how thoroughly parasitism has been demonstrated in experiments of the conventional type.

### CORRELATION OF INOCULATION RESULTS

The method of determining the results of inoculations has been to count and remove all damped-off seedlings, making the examinations every two or three days in the case of species like jack pine, which have small seed. Because of the extreme brittleness of the stems of young coniferous seedlings a certain number were accidentally broken in almost every experiment. These were removed as found, and recorded separately. The experiments were ordinarily closed from 10 to 20 days after fairly complete germination had been attained, in order to minimize the effect of accidental contamination on the results; and the surviving seedlings were counted. By adding the number of seedlings lost by damping-off and breakage to those surviving, the total number germinating was secured. By the number germinating is meant not the total germination but rather the number which developed far enough to break through the soil cover, this criterion being the only one it was practicable

to apply. The relative number of seedlings appearing in the inoculated pots and in the controls is an important element in the results, as it is a measure of the seriousness of the germination losses occasioned by the inoculations. It is, of course, not an exact measure because of the unavoidable differences in the number of seeds viable in the different pots at the time of sowing, but it is the only one available.

The effect of the inoculations in causing normal damping-off is best shown by the percentage of the germinated seedlings which damp-off. The absolute number damping-off is not a figure on which conclusions can be based, because of the great variations germination loss may cause in the initial stand. For example, if the pots inoculated with culture A had a germination of only 15 seedlings, the subsequent damping-off of 10 seedlings will mean much more than the damping-off of 20 seedlings in the pots inoculated with culture B, in which 100 seedlings may have originally germinated. As a large proportion of the seedlings lost by mechanical breakage are removed before the major part of the damping-off takes place, it is assumed that part of them would have damped-off had they not been broken. In view of this, the percentage of damped-off seedlings is obtained by the formula

$$\text{per cent damped-off} = \frac{D}{D+S} \times 100,$$

in which $D$ represents the number of seedlings damped-off, and $S$ the number surviving at the time the experiment was closed. The broken seedlings do not enter into this calculation.

The germination and the percentage of damping-off are of value as indicating the seriousness of the germination loss and of the normal damping-off, respectively, but neither figure represents the whole effect of the inoculation. The survival also is therefore given. If the limitations in its exactness due to accidental variations in germination are kept in mind, the comparative survival on inoculated pots and controls can be used as evidence as to the total effect of the inoculations both on germination loss and on normal damping-off. In order to free the survival figures, as far as possible, from the accidental variations due to the different amounts of loss by mechanical breakage in the different pots, it has been assumed that the broken seedlings in any series of pots, had they not been broken and removed, would have lost the same proportion of their number by damping-off as the unbroken seedlings. The actual survival is therefore in all cases adjusted by adding to it the number of the broken seedlings which the damping-off percentage indicates would have survived if they had not been broken. For example, in a set of pots in which the damping-off percentage is 40, the number of broken seedlings 15, and the surviving seedlings 56, the adjusted survival is obtained by adding to the actual survival 60 per cent of the number broken; the adjusted survival is thus 56+9, or 65. In most cases the

number it was necessary to add in adjusting the survival by this method was relatively much smaller than in this sample case.

In cases in which some of the experimental units contained more or larger pots than others, the germination and survival figures for the larger units were reduced proportionally, or were converted for all of the units to a percentage based on the number of seed sown, in order to permit the direct comparison of the figures for the different units.

## OOMYCETES CAUSING DAMPING-OFF

At least four oomycetes found in America appear capable of causing the damping-off of pine seedlings. Only one of these, *Pythium debaryanum* Hesse, is believed to be especially important on conifers. *Rheosporangium aphanidermatus* Edson, *Phytophthora* sp., and an apparent species of Pythium with spiny oogones, are the other oomycetes found attacking pines. As some of those concerned are of doubtful identity, the experimental results obtained with them will not be published until further studies now in progress on them have been completed.

## CORTICIUM VAGUM

Rhizoctonia was reported as a cause of the damping-off of white pine (*Pinus strobus*) in New York in 1901 (*6*). Preliminary inoculations on conifers were mentioned by the senior writer in an abstract published in 1910 (*13*). No inoculation evidence of its parasitism on conifers has ever been presented.

The strains of Rhizoctonia which were used successfully in the following experiments belonged to the common American Rhizoctonia, which causes the damping-off and rootrot of angiosperms, and is now usually referred to *Corticium vagum* B. and C. (identical with *C. vagum* var. *solani* Burt and with *Hypochnus solani* Prill. and Del.; *21, p. 286, footnote*). Spores have not been produced in any of the writers' cultures, but the identity of the Rhizoctonia on conifers with the common damping-off fungus on angiosperms is considered established by the following facts:

1. Widespread distribution and ready growth on various culture media of the strains from conifers. The only other common American Rhizoctonia, *R. crocorum* (*R. medicaginis; 21*) will not grow on ordinary artificial media (*4*).

2. Successful inoculations on pine seedlings with strains from, or which had been found parasitic on, dicotyledonous hosts.

3. Observed damping-off of dicotyledonous weed seedlings in patches coextensive with definitely limited damping-off patches of pine seedlings.

4. Successful inoculations by Edson (*7*) on dicotyledons with strains which the writers had taken from pines and had found parasitic on them.

Typical mycelium of *Corticium vagum* is easily demonstrated in the tissues of recently killed pine seedlings, and in the soil adjacent to them, and is obtained in culture by planting in solidified prune-agar plates small soil masses, or recently killed seedlings either with or without preliminary washing in mercuric-chlorid solution. Growth on the plates is very rapid, and transfers from the edges of the resulting colony give a large percentage of apparently pure cultures. A distinguishing character of the hyphæ, helpful in conjunction with the typical basal constrictions and septa, but less commonly mentioned, is the lack of tapering in young branches. Young hyphæ are nearly as large as older ones, with thick truncated tips, very unlike the fine tapering tips of some of the other soil fungi whose large hyphæ with basally constricted branches might otherwise be confused with young hyphæ of *C. vagum*.

The coniferous hosts from whose damped-off seedlings *Corticium vagum* has been isolated are western yellow pine[1] and jack pine from Nebraska, red pine (*P. resinosa*) from Minnesota and Michigan, Engelmann spruce from California and District of Columbia, and Douglas fir (*Pseudotsuga taxifolia*) from Colorado. Cultures from all of these hosts, with the exception of Douglas fir, have proved parasitic on pine seedlings. The single strain isolated from Douglas fir proved weakly if at all parasitic; so many infections have been observed in seedlings of this fir that it is believed some of them have been caused by parasitic strains. The fungus was also seen in the tissues of, or in cultures from, damped-off seedlings of Scotch pine (*Pinus sylvestris*), Corsican pine, Austrian pine (*Pinus austriaca*), and Norway spruce (*Picea excelsa*) without attempting to isolate it. With the previously reported findings of the fungus in damped-off white pine (*6*), yew (*Taxus cuspidata; 1*), and *T. canadensis*,[2] the number of coniferous hosts on which *C. vagum* apparently causes damping-off in nature is raised to 12, and will probably be much further increased when other conifers are studied.

Rhizoctonia has also been found associated with needle-killing of white pine (*2*) and Douglas fir (*14*) in seedlings more than 1 year old.

### INOCULATIONS ON AUTOCLAVED SOIL

Inoculations by the standard method described on page 526 were made with strains of *Corticium vagum* on conifers in the autumn of 1909, and repeated in later years. Inoculations with agar cultures broadcast at one side of the pot were made on jack pine in two experiments and on both red and western yellow pines in two other experiments, germination being reduced in the inoculated pots, and damping-off appearing, while the controls, with the exception of the red pine in the last experi-

[1] All of the western yellow pine mentioned in this paper was from seed collected in South Dakota, Colorado, or New Mexico, and is therefore the small-seeded form of *Pinus ponderosa* Laws (*P. scopulorum* Lemmon).

[2] The report of this latter species as a host for *Corticium vagum* is attributed to Clinton by Peltier (*21*, *p.* 304), though it does not seem to be mentioned by Clinton in (*1*).

ment, remained free from disease. With the last two hosts the controls received sterile agar.

In five additional experiments inoculum was distributed broadcast over the entire pot. In three of these, cultures on corn-meal mush were used as inoculum, and most of the germinating seed of jack and western yellow pine, and Douglas fir, respectively, were killed before they were able to break through the soil. The total number of seedlings involved in the experiment with Douglas fir was small. Though the controls were treated with sterile corn-meal mush and though the fungus was found in the killed seedlings in the inoculated flat, repetition is considered desirable for this host. In the two other experiments positive results were secured with both of the pines, with agar cultures as inoculum.

In five other experiments positive results were obtained with these pines by inoculating them with agar cultures at only one or two points in each pot and in one of them very definite results were also secured with white pine. In two of these experiments on jack pine, inoculation with single sclerotia in each pot was tried without the addition of any of the nutrient substratum with entire success. In the experiments with sclerotial inoculation and in the test on white pine the controls remained entirely free from disease. In the other experiments mentioned in this paragraph more or less accidental infection took place in the controls; positive results consisted in less germination and more subsequent damping-off in the treated plots than in the controls. In the inoculations mentioned in this and the preceding paragraphs the number of pots inoculated with *Corticium vagum* varied from a single 10-inch flat in the smallest experiment to 178 3-inch pots in the largest; the controls from a single flat to 30 pots.

In only two experiments *Corticium vagum* failed to give positive results. The loss was heavier in the inoculated pots than in the controls in both cases, but the difference was so slight as to be negligible. Both of these experiments involved inoculation at two points only in each pot.

These inoculations have the effect of confirming the field evidence of the parasitism of *Corticium vagum* on four pines, jack, red, white, and western yellow, and somewhat less positively on Douglas fir. Its apparent success on all of the coniferous hosts tested justifies the prediction that under favorable conditions it is likely to be found to be one of the causes of damping-off of most or all of the coniferous species which commonly suffer from this disease. Only part of the strains are vigorously parasitic on conifers, some strains, even though isolated from conifers, failing in repeated inoculation tests under favorable conditions to produce any considerable amount of disease.

## REISOLATION AND REINOCULATION

To complete the proof of the parasitism of *Corticium vagum* reisolations were made from recently killed seedlings of jack and western yellow pine in pots which had been inoculated with the fungus in an experiment in which the controls had remained free from disease. The results in the initial experiment and in two subsequent experiments conducted at different times in which the reisolated strains were used are given in Table I.

TABLE I.—*Inoculation with initial and reisolated strains of Corticium vagum on pine seedlings in autoclaved soil*

| Experiment No. and date. | Strain No. | Source. | Trial host. | Number of pots. | Location of inoculum. | Results. Germinated. | Results. Damped-off. | Results. Survival. |
|---|---|---|---|---|---|---|---|---|
| | | | | | | *Per 5 pots.* | *Per cent.* | *Per 5 pots.* |
| 58, fall, 1915. | 147 | Damped-off Engelmann spruce seedlings. | Western yellow pine. | 5 | Agar cultures at 1 point per pot. | 28 | 68 | 9 |
| Do... | ...do.. | .....do.............. | Jack pine..... | 5 | .....do.............. | 18 | 39 | 11 |
| Do... | [a] 213 | Damped-off sugar-beet seedlings. | .....do........ | 5 | Agar cultures at 6 points per pot. | 1 | 100 | 0 |
| Do... | 230 | Russian wild-olive seedlings.[b] | .....do........ | 5 | Agar cultures at 8 points per pot. | 4 | 100 | 0 |
| Do... | Control | ...................... | Western yellow pine. | 5 | None............... | 55 | 0 | 55 |
| Do... | ...do.. | ...................... | Jack pine..... | 10 | .....do............. | 78 | 0 | 78 |
| | | | | | | *Per 3 pots.* | | *Per 3 pots.* |
| 71 and 72, fall, 1917. | 147 | Damped-off Engelmann spruce seedlings. | Western yellow pine. | 2<br>3 | Agar cultures; fragments scattered over one side of pot. | 2<br>0 | 0<br>...... | 2<br>0 |
| Do... | 329 | Reisolation of No. 147 from western yellow pine, experiment 58. | .....do........ | 3<br>2 | .....do............. | 0<br>0 | ......<br>...... | 0<br>0 |
| Do... | 332 | .....do.............. | .....do........ | 3<br>2 | .....do............. | 3<br>0 | 100<br>...... | 0<br>0 |
| Do... | 333 | .....do.............. | .....do........ | 2<br>2 | .....do............. | 0<br>0 | ......<br>...... | 0<br>0 |
| Do... | 343 | Reisolation of No. 147 from jack pine, experiment 58. | .....do........ | 3<br>2 | .....do............. | 0<br>0 | ......<br>...... | 0<br>0 |
| Do... | [a] 213 | Damped-off sugar-beet seedlings. | .....do........ | 3<br>3 | .....do............. | 0<br>0 | ......<br>...... | 0<br>0 |
| Do... | 340 | Reisolation of No. 213 from jack pine, experiment 58. | .....do........ | 2<br>3 | .....do............. | 3<br>0 | 100<br>...... | 0<br>0 |
| Do... | 233 | Russian wild-olive seedlings [b] (duplicate of No. 230). | .....do........ | 1<br>2 | .....do............. | 9<br>16 | 0<br>8 | 9<br>16.5 |
| Do... | 330 | Reisolation of No. 230 from jack pine, experiment 58. | .....do........ | 1<br>3 | .....do............. | 9<br>8 | 0<br>0 | 9<br>8 |
| Do... | 341 | .....do.............. | .....do........ | 3<br>3 | .....do............. | 1<br>6 | 100<br>33 | 0<br>4 |
| Do... | Control | ...................... | .....do........ | 4<br>14 | Sterile agar fragments scattered over one side of pot. | 9<br>9 | 0<br>0 | 9<br>9 |
| Do... | 147 | Damped-off Engelmann spruce seedlings. | Red pine...... | 2<br>2 | Agar culture fragments scattered over one side of pot. | 6<br>0 | 100<br>...... | 0<br>0 |

[a] Cultures furnished by Dr. H. A. Edson, Bureau of Plant Industry.
[b] Diseased material furnished by Mr. B. R. H. d'Allemand.

TABLE I.—*Inoculation with initial and reisolated strains of Corticium vagum on pine seedlings in autoclaved soil*—Continued

| Experiment No. and date. | Strain No. | Source. | Trial host. | Number of pots. | Location of inoculum. | Results. Germinated. | Results. Damped-off. | Results. Survival. |
|---|---|---|---|---|---|---|---|---|
| | | | | | | *Per 3 pots.* | *Per cent.* | *Per 3 pots.* |
| 71 and 72, fall, 1917. | 329 | Reisolations of No. 147 from western yellow pine, experiment 58. | Red pine..... | 2<br>3 | Agar culture fragments scattered over one side of pot. | 14<br>2 | 11<br>100 | 12<br>0 |
| Do... | 332 | .....do.............. | .....do........ | 3<br>3 | .....do............. | 2<br>0 | 100<br>...... | 0<br>0 |
| Do... | 333 | .....do.............. | .....do........ | 2<br>3 | .....do............. | 8<br>0 | 100<br>...... | 0<br>0 |
| Do... | 343 | Reisolation of No. 147 from jack pine, experiment 58. | .....do........ | 3<br>3 | .....do............. | 2<br>0 | 0<br>...... | 2<br>0 |
| Do... | [a] 213 | Damped-off sugar-beet seedlings. | .....do........ | 3<br>3 | .....do............. | 5<br>0 | 100<br>...... | 0<br>0 |
| Do... | 331 | Reisolations of No. 213 from jack pine, experiment 58. | .....do........ | 2<br>2 | .....do............. | 3<br>0 | 100<br>...... | 0<br>0 |
| Do... | 340 | .....do.............. | .....do........ | 3<br>3 | .....do............. | 4<br>1 | 75<br>100 | 1<br>0 |
| Do... | 230 | Russian wild-olive seedlings. | .....do........ | 3<br>2 | .....do............. | 58<br>33 | 2<br>5 | 57<br>31.5 |
| Do... | 330 | Reisolations of No. 230 from jack pine, experiment 58. | .....do........ | 3<br>3 | .....do............. | 65<br>41 | 3<br>0 | 63<br>41 |
| Do... | 341 | .....do.............. | .....do........ | 3<br>2 | .....do............. | 31<br>30 | 45<br>30 | 17<br>30 |
| Do... | Control | ...................... | .....do........ | 5<br>16 | Sterile agar fragments scattered over one side of pot. | 58<br>35 | 0<br>5 | 58<br>33 |

[a] Cultures furnished by Dr. H. A. Edson, Bureau of Plant Industry.

It is evident from the results in Table I that the reisolated cultures were able to cause germination loss or damping-off, or both, in both the hosts on which they were tried. The approximate agreement in virulence in experiment 71 and 72 of the original strains and the strains isolated from the pots inoculated with them is an additional evidence that the strains recovered were the ones originally used. Strain 341 seemed rather more virulent than 230 from which it was reisolated; it was, nevertheless, obviously less active as a parasite than strains 147 and 213, and their reisolations. The possibility that a strain of *Corticium vagum* obtained from an autoclaved and inoculated pot is not a true reisolation of the strain used in the initial inoculation is very slight, in view of the lack of adaptation of the fungus to aerial dissemination and the fact that in the writers' numerous cultures from autoclaved soil experiments, *C. vagum*, unlike *Fusarium* spp. and *Pythium debaryanum*, has never been detected in pots in which it had not been intentionally introduced. As the cultures used were all, or nearly all, obtained by the planted-plate method, their purity is not entirely beyond question. However, their apparent purity, continued in the case of strain 147 through eight years of growth on artificial media, and the permanence of the relative

virulence of most of the strains through several years cultivation, indicate not only that the cultures used contained only *C. vagum*, but that most of them contained only a single strain of the fungus.

On the whole, the limited comparisons possible of initial and reisolated strains, do not indicate any decided increase in virulence on pine seedlings as a result of a single passage through the host.

## CROSS-INOCULATIONS

In Table I is presented evidence of parasitism on three species of pine of strains of *Corticium vagum* from spruce and sugar beet. In one of these experiments and also in an earlier experiment not included in this table, the original strains from Russian wild olive (*Elaeagnus sp.*), proved decidedly parasitic on jack pine. The strains 147 and 213, from spruce and sugar beet, respectively, have proved more virulent on the pines in these and other experiments than any of the strains coming originally from pine seedlings. The slight ability of a single strain from Douglas fir to infect pine, referred to earlier, does not prove any specialization of virulence, as some of the strains from pine give negative results on the same species of pine unless given very favorable working conditions. The positive results on Douglas fir with a strain from jack pine in a single very small-scale test made is indication of the lack of any specialization of strains of *C. vagum* to pine or Douglas fir.

Inoculations on both jack and western yellow pine have been successful with strains from potato tuber and from bean stems, the latter culture being furnished by Dr. M. F. Barrus, who stated that it was from a strain of proved parasitism on beans. The strains from these two hosts were parasitic on pines only under very favorable conditions. Single experiments with strains from alfalfa and carnation supplied by Dr. Barrus, indicated that these were only weakly, if at all, parasitic on pine seedlings. A strain from a sugar-beet root, isolated in eastern Colorado by Dr. F. A. Wolf, of the North Carolina Experiment Station, proved moderately parasitic on pine seedlings, while another strain from the same host and locality showed little, if any, virulence on pine even under the most favorable conditions. All three of the sugar-beet strains had been previously tested by Dr. Edson in his inoculations on sugar-beet seedlings, and found parasitic on them. Cultures from western yellow pine were also tested on sugar-beet seedlings by Edson (7) with positive results. A strain from jack pine, only moderately parasitic on that pine species in inoculation, was found by Dr. Barrus to produce lesions on bean stems, though small and atypical as compared with his own strains.

The inoculation evidence, as a whole, supports the conclusion that the Rhizoctonia causing the damping-off of pines is the same as the Rhizoctonia commonly concerned in the seedling disease of dicotyledons. The same strain can cause disease of both conifers and dicotyledons. Cer-

tain strains may be slightly specialized to particular hosts, but such variations are hardly of taxonomic value.

INOCULATIONS ON UNSTERILIZED SOIL

In an experiment in which a very sandy western Kansas soil was treated with 0.5 fluid ounce of sulphuric acid per square foot, followed two days later by 0.9 ounce of air-slaked lime per square foot, a number of strains of *Corticium vagum* were tested, inoculum on cooked rice being distributed through the drills. Most of the strains either prevented or greatly decreased the germination of both jack and western yellow pines, and the more virulent strains caused the damping-off of practically all of the seedlings which were able to get through the soil surface. In the plots inoculated with strains of *C. vagum* which had indicated any decided degree of virulence in earlier tests the survival ranged from 0 to 47 seedlings (44 linear inches of drill, half of each pine, for each fungus strain), while the 16 controls in the same experiments ranged from 59 to 254 seedlings for equal lengths of drill, more than half of the controls having survivals better than 150 seedlings. The most virulent strain of *Pythium debaryanum* heavily inoculated in the same way and at the same time resulted in little more parasitic loss than occurred in control plots inoculated with saprophytic molds or with nothing at all. The inoculations with *Corticium vagum* caused as much damping-off as would normally be secured by inoculating autoclaved soil under the most favorable conditions for parasitism. The advantage of the *C. vagum* over the *Pythium debaryanum* in this case was probably at least in part due to the lack of humus. *P. debaryanum* apparently prefers soils with a reasonable amount of humus, while *C. vagum* has been frequently found in damped-off seedlings on this same soil and on another very sandy, humus-poor soil.

In an experiment in which a heavier soil heated in a moist condition to a temperature of 80° to 90° C. for a period of not less than 10 minutes was inoculated with the parasites. *Corticium vagum* again caused marked decreases in germination, while *Pythium debaryanum* had little effect on the number of seedlings which appeared. Outside infections, probably with *Fusarium* spp., which it was impossible to exclude in this outdoor work, destroyed the seedlings so rapidly after germination that it was impossible to obtain evidence on the effect of the inoculum on the seedlings after they came up. The definite superiority of *C. vagum* over the *P. debaryanum* in causing germination loss in this case can not be attributed to lack of humus.

In soil entirely untreated, *Corticium vagum* was used in two very small-scale inoculation experiments in the Washington greenhouse, inoculum being placed at one and two points in each pot, respectively. In both cases *C. vagum* definitely decreased the survival. In the first experiment *Pythium debaryanum* had no effect, and in the second affected only germination, while *C. vagum* both decreased germination and increased

subsequent damping-off. The soil used was a greenhouse mixture of sand and loam.

FUSARIUM SPP.

Species of Fusarium and Fusoma have been frequently reported as being the cause of damping-off of conifers in Europe. The first crude inoculations were made by Hartig (*12*), who produced typical damping-off by placing healthy seedlings in contact with plants which had damped-off and were bearing spores of *Fusarium* spp. Von Tubeuf (*27*) reports having inoculated pine seedlings with artificial cultures, but in so small an experiment and with so little in the way of positive results as to be inconclusive. He later (*27*) states that he and Hartig have repeatedly caused the death of plants of European conifers by inoculating them with *Fusarium parasiticum*, "also from pure cultures." No detailed report of pure-culture inoculation experiments has been furnished in the European literature noted, and the ability of the Fusarium or Fusoma strains, which have been variously mentioned under the specific names of *pini*, *parasiticum*, and *blasticola* to cause damping-off in Europe has rested mainly on the frequency with which they have been obtained from damped-off conifers. The strains which have been found on conifers and which have been used in inoculation are not sufficiently well described to make it possible to connect them with any of the species at present recognized. There is furthermore no way of telling whether the different reports of species of Fusarium and Fusoma on coniferous seedlings refer to the same or different organisms.

In America Spaulding (*25*) reports briefly inoculation experiments with a number of different strains of Fusarium the detailed record of which the writers have been permitted to examine. Sufficient damping-off occurred in some of the control plots, so that it does not seem possible to say for any one of the strains he used that its parasitism was proved, in view of the fact that each strain was used in a single pot only. For the strains in general, his work established beyond reasonable doubt that at least some of them were parasitic, the loss in the pots inoculated with *Fusarium* spp. as a whole averaging well above that in the controls. Among the species for which parasitism was indicated quite strongly are *F. vasinfectum* E. F. Smith and *F. moniliforme* Sheldon. The evidence is especially strong for the latter species. *F. vasinfectum* from cotton gave apparently more positive results than the strain from watermelon. The general conclusion from his experiment seems to be that a number of different strains or species of Fusarium are probably able to attack pine seedlings under the very favorable inoculation conditions which he furnished, and that *F. moniliforme* is one of the more virulent.

Prof. P. S. Lovejoy, working as a student under the direction of Dr. J. B. Pollock in 1907, two years after Spaulding did his work, produced damping-off in western yellow pine with a species of Fusarium isolated from

damped-off pine seedlings. His experiments were conducted in autoclaved earth, with several pots both inoculated and controls, all being under bell-jars. The controls remained free from disease. His species of Fusarium, which he called *F. pini*, can not be positively identified with any of the species at present recognized, from the data given in his unpublished manuscript, which he kindly allowed the writer to examine. A species of Nectria (later reported by Pollock; *22*) was associated with the species of Fusarium.

Gifford also carried out inoculation experiments with species of Fusarium indicating its parasitism. His statement (*9, p. 157*) seems reasonably conclusive, though the tables on the pages preceding and following, presumably by reason of typographical errors, do not bear out his statement as to the original disease freedom of his control pots. The host with which he experimented was Scotch pine. The description of the fungus which he gave indicates that it is not *F. moniliforme*, but does not make it possible to refer it to any of the other recognized species. While his inoculations were on autoclaved soil, the fungus was not given quite such optimum conditions as in Spaulding's work, in that his inoculum consisted of a spore suspension without any considerable amount of nutrient medium accompanying.

### FUSARIUM MONILIFORME

The species of Fusarium which the writers have found most virulent among the four or more species which they have tested is *F. moniliforme*[1] This fungus is fortunately easy to distinguish from other species of the genus, as it is understood to be the only species of Fusarium, in the United States at least, which forms its microspores in chains. It is presumably not identical with the species and relatives of Fusarium described as troublesome to coniferous seedlings in Europe, as none of the European reports noted mentioned moniliform spores. The microspores in the writers' cultures were ordinarily produced in delicate long unbranched chains. Presumably on account of the delicacy of these spore chains, the fungus seems especially well adapted for aerial dissemination. In planted petri-dish cultures numerous new colonies usually start well in advance of the original colony, before the mycelium from the original inoculation is able to cross the plate. In some cases, as in Sheldon's cultures (*23*), microspores were agglomerated into heads, and occasionally small heads of spores with chains arising from them have been observed. The spores in the chains measured in one case from a prune-agar culture 2.2 to 3.1 by 4.8 to 6.3 μ. In another case from a corn-meal agar culture 27 of the microspores ranged in length from 7.4 to 11.1 μ. Many of the cultures on the above media, but not all, developed

[1] All positive Fusarium identifications were made by Mr. C. W. Carpenter, Plant Pathologist of the Hawaiian Experiment Station, based on comparisons with stock cultures named by Dr. W. H. Wollenweber, formerly of the Bureau of Plant Industry.

a characteristic grape-juice purple, which diffused through the agar. This was not constant even for the same strain. The moniliform character of the microspores of this species can be very easily demonstrated by growing the fungus on a thin layer of nutrient agar in a petri dish and examining the culture from above with the compound microscope after four or five days' incubation at room temperature.

The cultures used by the writers were isolated from seedlings of jack and western yellow pine from nurseries on somewhat alkaline and very sandy soils in southwestern Kansas. It is not believed to be a common organism on pine seedlings in most localities.

One or more strains of *Fusarium moniliforme* were tested in five inoculation experiments on jack pine in autoclaved soil. In two of these experiments, inoculation was only light or moderately heavy, and the pots inoculated with *F. moniliforme* suffered distinctly less from damping off than the controls, which were rather seriously affected as a result of accidental contamination. A virulent strain of *Corticium vagum* also proved a failure in one of the experiments, and the most virulent strains of Corticium and Pythium were only slightly active in the other. The results in the three remaining experiments, together with the results from all the other fungi which were used in the same experiments, are given in Table II, with the exception that the results of inoculation with strains of Corticium, which were atypical or intermediate in virulence, in experiment 31 are omitted. The results of the two larger experiments seem to establish beyond any serious doubt the ability of *F. moniliforme* to cause damping-off of jack pine on autoclaved soil when sufficient inoculum is added. Absolute final proof must, of course, await compliance with Koch's postulates and should be based on inoculation with single spore cultures. The cultures used in these experiments were all from planted plates. Nevertheless all of those whose results are reported were apparently pure, and in view of the number of strains and of controls in experiments 31 and 60, the parasitism of the fungus under favorable conditions is considered practically established. In addition to the pots formally designated as controls, the 35 pots inoculated with *Trichoderma* sp. and with unidentified or mixed cultures in experiment 31 serve perhaps as still better controls, as they received in the inoculum the same nutrient medium as was applied to the pots inoculated with *F. moniliforme*.

TABLE II.—*Inoculation with Fusarium spp. and other fungi on jack pine in autoclaved soil*

| Experiment No. and date. | Nutrient substratum introduced with inoculum. | Location of inoculum. | Number of pots. | Fungus. | Germination (percentage of seed). | Damped-off (percentage of seedlings). | Survived (percentage of seed). |
|---|---|---|---|---|---|---|---|
| 31, fall, 1913. | Prune agar and steamed rice. | Broadcast throughout pot. | 5 | *F. moniliforme* (strain 239). | 44.1 | 36.0 | 28.8 |
| Do | do | do | 5 | *F. moniliforme* (strain 249). | 35.8 | 48.0 | 18.6 |
| Do | do | do | 5 | *F. moniliforme* (strain 251). | 46.0 | 90.0 | 4.7 |
| Do | do | do | 5 | *F. moniliforme* (strain 260). | 49.3 | 53.0 | 23.7 |
| Do | do | do | 5 | A species of Fusarium somewhat like *F. solani* (strain 235). | 41.5 | 9.0 | 38.0 |
| Do | do | do | 5 | A weak strain of *Pythium debaryanum* (258) | 36.9 | 18.0 | 30.6 |
| Do | do | do | 5 | A virulent strain of Corticium (147). | 2.9 | 73.0 | .7 |
| Do | do | do | 5 | *Trichoderma* sp. (252) | 51.1 | 2.7 | 49.5 |
| Do | do | do | 30 | Unidentified or mixed cultures (strains 244, 250, 236, 238, 246, 248). | 48.1 | 4.6 | 45.9 |
| Do | Steamed rice | do | 5 | *F. ventricosum* | 40.0 | 38.0 | 25.2 |
| Do | Corn-meal agar and prune agar. | do | 5 | *F. solani* | 45.4 | 14.0 | 39.0 |
| Do | Corn-meal agar | do | 5 | *F. acuminatum* | 55.5 | 2.2 | 54.2 |
| Do | do | do | 5 | *Rheosporangium aphanidermatus* (229)[a]. | 45.3 | 13.0 | 40.0 |
| Do | do | do | 5 | *Phoma betae* [a] | 48.6 | 5.6 | 45.9 |
| Do | do | do | 5 | *Chaetomium* sp. | 45.7 | 6.2 | 42.8 |
| Do | do | do | 5 | *Trichothecium roseum* | 48.2 | 3.4 | 46.3 |
| Do | Prune agar | do | 5 | A virulent strain of *P. debaryanum* (255). | 12.4 | 44.0 | 6.8 |
| Do | do | do | 10 | The most virulent strains of Corticium (213,[a] 233). | 3.9 | 60.0 | 1.3 |
| Do | do | do | 10 | The 2 least virulent strains of Corticium (205, 186). | 44.5 | 4.7 | 42.4 |
| Do | do | do | 5 | *Phoma* sp. | 50.0 | 3.4 | 48.3 |
| Do | None | | 25 | Controls | 43.0 | 5.2 | 40.8 |
| 57, fall, 1914. | Autoclaved Melilotus stems. | Spore suspension after sowing and 5 small pieces of stem inoculum in pot at germination. | 8 | *F. moniliforme* (249) | 37.7 | 5.3 | 35.8 |
| Do | Corn-meal agar | 2 points at one side of pot. | 7 | *P. debaryanum* (295) | 38.8 | 1.8 | 38.2 |
| Do | do | do | 5 | *Corticium vagum* | 37.3 | 10.3 | 33.5 |
| Do | None | | 12 | Controls | 42.0 | .4 | 41.8 |
| | | | | | *Per 5 pots.* | *Per 5 pots.* | *Per 5 pots.* |
| 60, 1915 | Prune agar | 1 point at edge of pot. | 5 | *F. moniliforme* (260) | 54.0 | 6.0 | 51.0 |
| Do | do | 4 points in pot | 5 | do | 50.0 | 32.0 | 34.0 |
| Do | do | Broadcast at one side of pot. | 5 | do | 28.0 | 57.0 | 12.0 |
| Do | do | Broadcast throughout pot. | 5 | do | 40.0 | 35.0 | 26.0 |
| Do | Steamed rice | do | 10 | do | 22.5 | 42.0 | 13.0 |
| Do | Prune agar and steamed rice. | do | 5 | do | 4.0 | 50.0 | 2.0 |
| Do | None (spore suspension). | do | (1st unit) 5. | do | 46.0 | 4.3 | 44.0 |
| Do | do | do | (2d unit) 5. | do | 51.0 | 43.0 | 29.0 |
| Do | Prune agar | 1 point at edge | 5 | *Fusarium* sp. (273) | 59.0 | 5.1 | 56.0 |
| Do | do | 4 points in pot | 5 | do | 47.0 | 4.3 | 45.0 |
| Do | do | Broadcast at one side of pot. | 5 | do | 53.0 | 1.9 | 52.0 |
| Do | do | Broadcast throughout pot. | 5 | do | 44.0 | 18.0 | 36.0 |
| Do | Steamed rice | do | 10 | do | 46.0 | 21.0 | 34.5 |

[a] Furnished by Dr. H. A. Edson.

TABLE II.—*Inoculation with Fusarium spp. and other fungi on jack pine in autoclaved soil*—Continued

| Experiment No. and date. | Nutrient substratum introduced with inoculum. | Location of inoculum. | Number of pots. | Fungus. | Germination (percentage of seed). | Damped-off (percentage of seedlings). | Survived (percentage of seed). |
|---|---|---|---|---|---|---|---|
| 60, 1915.. | Prune agar and steamed rice. | Broadcast throughout pot. | 5 | *Fusarium* sp. (273).... | 33.0 | 15.0 | 18.0 |
| Do.... | None (spore suspension.) | .....do.............. | (1st unit) 5. | .....do.................. | 49.0 | 6.0 | 46.0 |
| Do.... | .....do................ | .....do.............. | (2d unit) 5. | .....do................ | 58.0 | 1.7 | 57.0 |
| Do.. . | Prune agar and steamed rice. | .....do.............. | 5 | *F. solani* (202)........ | 20.0 | 40.0 | 12.0 |
| Do.... | None................ | ..................... | (1st unit) 5. | Control (*a*)............ | 57.0 | 7.0 | 53.0 |
| Do.... | .....do................ | ..................... | 2d unit) 5. | Control (*b*)............ | 55.0 | 7.0 | 51.0 |
| Do.... | .....do................ | ..................... | (3d unit) 5. | Control (*c*)........ | 48.0 | 0.0 | 48.0 |
| Do.... | .....do................ | ..................... | All. | Control (*a, b, c*)....... | 53.3 | 5.0 | 50.7 |

The peculiar behavior of *Fusarium moniliforme* in actually seeming to prevent part of the damping-off in the first two experiments mentioned (the two which are not included in Table II) suggests the possibility that in autoclaved soil under circumstances in which it is not itself able to act as a parasite, it may nevertheless by reason of its vigorous saprophytic growth so occupy the soil as to make it a less favorable medium for the spread of the more virulent parasite or parasites whose presence was indicated by the considerable damping-off in the controls in these experiments. That competition of saprophytic fungi may limit the damage done by *Pythium debaryanum* in recently autoclaved soil is indicated by the results of counterinoculation experiments which will soon be published. Competition between parasites, or between a parasite and a potentially parasitic fungus under conditions which render the latter nonparasitic, is quite probable. In the present case the record of this species of Fusarium in killing other fungi in mixed cultures in agar adds color to the possibility that it may effectively hinder more virulent parasites in autoclaved soil.

In experiment 60 it appears that in heavy inoculations with cultures on nutrient substrata *Fusarium moniliforme* caused germination loss as well as damping-off after the seedlings appeared. This agrees with Spaulding's conclusions (*26*). It is apparently only under exceptionally favorable conditions such as very heavy inoculation, or, as in Spaulding's work, unusually deep sowing, that serious germination loss is to be expected from this species of Fusarium. Comparison of the Fusarium pots inoculated with *F. moniliforme* with those inoculated with virulent strains of

Pythium and Corticium in experiment 31 indicates much less damage by *F. moniliforme* to germinating seed. While some of the strains of Corticium and Pythium also do little or no damage to germinating seed in inoculations by the writer's standard methods, they are in most cases strains whose general virulence is low, and which are not able to kill many seedlings after germination. The indications are that *C. vagum* is rather better able to kill germinating seed than seedlings which have recently appeared above the soil; that *P. debaryanum* is at least approximately as well able to cause germination loss as normal damping-off; and that *F. moniliforme* is distinctly less able to cause germination loss than it is to cause subsequent damping-off.

The relation of heavy inoculation to positive results with *F. moniliforme* is indicated, first, by the negative results in the two nontabulated experiments in which inoculation was light, the weakly positive results in experiment 57 in which inoculation was fairly heavy, and the unquestionable results in the heavier inoculations in experiments 31 and 60; and second, by the difference in results between the heavily inoculated and the parallel lightly inoculated pots in experiment 60. It is not possible to say whether the increased damping-off in the heaviest inoculations was simply due to the development in the soil of a larger amount of mycelium and consequently more points of contact between hyphæ and seedlings, or whether in the cases in which large amounts of nutrient substratum were added with the fungus, there was an actual temporary increase of virulence resulting. It appears from experiment 60 that broadcast inoculations with spore suspensions were distinctly more effective than inoculation with a small fragment of a culture at a single point at the edge of each pot. The single point inoculations resulted in no more damping-off than occurred in the controls. Broadcast inoculation over the entire pot and including nutrient substrata were definitely successful in all four of the 5-pot units on which it was used. These heavy inoculations were clearly more effective tlan the inoculations over smaller areas or those made with spore suspensions. In comparing these heavily inoculated units with each other and considering both germination loss and subsequent damping-off, it appears that inoculation with cultures on steamed rice is more effective than with cultures on prune agar, and that the greatest total loss occurred following the use of both media in the inoculum. The apparent increase in damping-off with increase in the amount and richness of the media, though by no means final proof, is believed to indicate that the presence of the nutrient substrata actually increased the virulence of the parasite, as well as assisting it to become thoroughly distributed throughout the pot. The possibility that the substrata added may have decreased the resistance of the seedlings must also, of course, be considered.

In the series of outdoor inoculations on a Kansas sand lacking humus and treated with sulphuric acid followed by lime, mentioned in the foregoing account of *Corticium vagum*, three strains of *Fusarium moniliforme* were tested, each being added to 2 plots of jack pine and 2 plots of western yellow pine. The average germination for all 12 inoculated plots was only 0.7 that in the 16 nearest control plots, and subsequent damping-off was slightly greater than in the controls. The net results indicate much less parasitism by *F. moniliforme* under these conditions than by most of the Corticium strains used in the same experiment, although the species of Fusarium appears nearly or quite as parasitic as the cultures of *Pythium debaryanum* used. The variation in the controls in the parts of the experiments containing the Fusarium plots was unusually great, and the results less conclusive than those for *P. debaryanum*.

The virulence of different strains of *Fusarium moniliforme* apparently differs (Table II, experiment 31), though the range of variation is less for the four strains worked with than was found to be the case in the larger number of strains of *Pythium debaryanum* and *Corticium vagum*. The weakest strain was more virulent than the weakest strains of these two fungi, but the most virulent strains of *F. moniliforme* were considerably less destructive to jack pine than the most virulent strains of the two better known parasites, at least on the youngest seedlings. No direct comparison of virulence is possible between three fungi whose different strains vary so decidedly among themselves, unless large numbers of strains of all three from different sources are studied. It seems safe, however, to say that *F. moniliforme* is distinctly less important than *P. debaryanum* or *C. vagum* as a parasite on pine seedlings, in view of its apparent infrequence at most nurseries and its failure to cause serious damping-off in inoculation tests, except with very heavy inoculations.

### FUSARIUM VENTRICOSUM

A species of Fusarium obtained in association with *F. moniliforme* on an apparently healthy root of western yellow pine was identified by Mr. C. W. Carpenter as *F. ventricosum* Appel and Wollenw. In inoculation on jack pine (Table II, experiment 31) the fungus showed decided evidence of parasitism. Like *F. moniliforme*, in the same series, it had no marked effect on germinating seed, but caused subsequent damping-off loss more than seven times as great as that which occurred in the uninoculated pots, and eight times as great as that in pots inoculated with various saprophytic organisms on the same nutrient medium. There seems little question as to its parasitism, though further tests, involving single-spore cultures and reisolation, will be needed for final proof. How frequently it occurs in pine seed beds is not known; it is, however, believed not to be especially common. Its indicated virulence in the test mentioned was a little below that of *F. moniliforme*.

FUSARIUM SOLANI

A species of Fusarium obtained by Mrs. H. E. Watkins from damped-off western yellow pine in Nebraska was determined by Mr. C. W. Carpenter as *F. solani.* In a single-pot test on jack pine in autoclaved soil it gave some indication of parasitism, but very much weaker than any of the *Pythium debaryanum* strains used in the same experiment. In a later experiment (No. 31, Table II) it gave definite indication of a slight degree of parasitism, both on comparison with the uninoculated controls and with the numerous pots inoculated with species of Phoma, Trichothecium, Trichoderma, Chaetomium, nonvirulent Corticium strains, and other fungi, in all of which damping-off was less than half that in the *F. solani* pots. Though comparison with the other parasites is somewhat uncertain because of the different nutrient substrata used in the inoculum, *F. solani* appears distinctly less parasitic than the stronger strains of Pythium and Corticium (No. 255, 147) and those of *F. moniliforme* and *F. ventricosum* tested, while about equal in effectiveness to a weak Pythium strain and to *Rheosporangium aphanidermatus*. In experiment 60 of Table II heavy inoculation with *F. solani* resulted in decided germination loss and subsequent damping-off, though less than that indicated for *F. moniliforme* with the same inoculum. The absence of nutrient substratum in the control pots prevents the experiment being quite as conclusive as it might have otherwise been.

In inoculation tests on jack pine and western yellow pine on soil treated with acid and lime, referred to in preceding sections, it failed to affect either germination or subsequent damping-off, while all but the very weakest strains of Corticium caused greatly increased losses, and *Pythium debaryanum* and *Fusarium moniliforme* appeared to have moderately increased loss.

The experiments as a whole indicate that the strain of *Fusarium solani* used in these experiments is a weak parasite on jack pine. Despite the relatively slight virulence of this strain, this species seems worth serious consideration as a damping-off organism, in view of its widespread occurrence.

OTHER SPECIES OF FUSARIUM

A preliminary test was made of a mixture of three of Spaulding's cultures of *Fusarium* spp. and three obtained by the writers from pine seedlings at Halsey, Nebr., the latter superficially resembling *F. solani* in heavy inoculation with rice inoculum on jack pine on autoclaved soil and under moist chamber conditions. Damping-off was negligible. In the first test mentioned under *F. solani*, another strain of Fusarium resembling it was also tested on a single pot, with less resultant damping-off than in the control pots; three strains of *Pythium debaryanum* in the same series all caused heavy and unmistakable increases in damping-off.

In experiment 31, Table II, another strain of Fusarium superficially resembling *F. solani* was tested in heavy inoculation on five pots of jack pine. Damping-off after germination was nearly double that in the uninoculated controls and quite double that in pots inoculated with saprophytic molds on the same substrata. The loss, however, was only two-thirds as much as in the pots inoculated with *F. solani*, *Rheosporangium aphanidermatus*, and weak *Pythium debaryanum*, and much less than in the pots inoculated with the other parasites. The same culture, tested later in the aforementioned experiments on soil treated with acid and lime, gave no indications of parasitism on western yellow pine and little, if any, on jack pine. In all the experiments with this culture the difference between the inoculated pots and the controls was easily within the limits of accidental variation.

A species of Fusarium obtained from damped-off seedlings of western yellow pine in a greenhouse at Washington, D. C., and identified by Mr. Carpenter as *F. acuminatum* E. and E., was tested in experiment 31, Table II, with less subsequent damping-off than in the controls. In the experiment on acid-lime-treated soil it had no apparent effect on yellow pine and on jack pine seemed to increase the amount of damping-off after germination but not more than might be explained on the basis of accidental variation.

A culture of *Fusarium* sp. of uncertain identity (No. 273, experiment 60, Table II) gave positive inoculation results in very heavy inoculations only. This culture was recorded in early notes as producing microspores in chains, but it was impossible to confirm this in subcultures made some time later. In the experiment this culture appeared not only much less virulent than the *F. moniliforme*, but even less so than the *F. solani* culture tested. In view of its doubtful identity and purity, the results with it are of interest chiefly in its agreement with the results in the pots of *F. moniliforme*, broadcast inoculation proving more effective than inoculation over limited areas, agar-culture inoculum proving more effective than spore suspensions, and rice inoculum appearing perhaps still more effective, while the very heavy inoculation involved in the use of both media had the maximum effect.

The conclusion to be drawn from these somewhat fragmentary data is that the strains of *Fusarium moniliforme* and *F. ventricosum* experimented with were above the average of virulence for species of Fusarium on pine seedlings, and that finding a species of Fusarium on damped-off seedlings does not establish as strong a presumption of etiological significance as would the finding of *P. debaryanum* or *C. vagum*. *F. solani* and *F. vasinfectum* also appear somewhat parasitic on pine seedlings, and *F. acuminatum* nonparasitic. Further tests with some of these and other authentically identified species of Fusarium are badly needed to settle the question as to which species are capable of parasitism and

what is their importance as compared with the parasites belonging to other genera, under natural soil conditions.

## TRICHODERMA.

Strains of Trichoderma frequently appear in cultures from seedlings affected with the usual type of damping-off. They also often fruit on the surface of recently autoclaved soil, entirely independently of the presence of seedlings. Strains isolated at a Kansas nursery produced conidiophores in very compact groups, often as much as 2 mm. in diameter, and usually arranged in zonate rings. The spores were mostly spherical. This agrees with the characters given for *T. lignorum* (Tode) Harz., though one of the zonate strains isolated had the elliptical spores described by Cook and Taubenhaus (*3*) for *T. köningi* Oudemans, and another, with spherical or nearly spherical spores, showed the absence of zonate spore-tuft production attributed to *T. köningi*. Frequent chlamydospore formation, described as characteristic of *T. köningi*, was observed in some of the strains whose isolation was not attempted. It appears that both species, if they are to be regarded as distinct species, are more or less common in pine seed beds.

In an early inoculation test with an unidentified strain of Trichoderma from Washington, D. C., an autoclaved flat was sown with jack pine, inoculated broadcast, and kept under moist-chamber conditions without result. In a later experiment (No. 31, Table II) with the elliptical-spored strain above referred to, the nonzonate strain, and the more typical *Trichoderma lignorum* strain, in cultures of uncertain purity, were used in heavy inoculation on five pots each with actually less parasitism than occurred in the controls. A fourth culture of *Trichoderma* sp. of doubtful purity resulted in somewhat increased damping-off, but within the limit of experimental error.

In the experiment on sand treated with acid and lime, referred to in the accounts of experiments with the preceding fungi, one pure and two doubtfully pure cultures representing the three different types were tested. The most typical strain of *Trichoderma lignorum* had no effect on western yellow pine and little or none on jack pine. The nonzonate form and the elliptical-spored form both appeared to decrease germination in both pines, the former also increasing damping-off in western yellow pine and the latter in jack pine. The differences were in all cases within the limits of accidental variation. The indications from these tests are that the strains of Trichoderma used are either unable to cause the usual type of damping-off or are very unimportant causes. The frequency with which the fungus is obtained in planted-plate cultures from dead seedlings may easily be due to its common presence in soil and its capacity for rapid growth on the agar used.

In addition to the usual type of damping-off, the blacktop type, mentioned in the first part of the paper and illustrated in Plate B, figures 2 and

3, is to be considered in connection with Trichoderma. Plate cultures from 30 jack-pine seedlings affected with blacktop in a nursery in the Nebraska sand hills in every case yielded species of Trichoderma; no such uniformity of occurrence has been encountered for any fungus in any of the series of cultures made from other types of damping-off.

The blacktop type occurs rarely, and, so far as observed, only under unusual weather conditions, most of that observed having followed unseasonably cold, wet weather. Extensions of the lesions into unaffected tissue stopped simultaneously and abruptly throughout the beds, apparently because a change in conditions increased resistance. The affected seedlings were scattered throughout the beds, seeming equally common on acid-treated and untreated areas. The entire picture was that of a disease caused by a fungus which is not strongly parasitic, and is well adapted for aerial dissemination. *Trichoderma* sp. seems to fulfill both of these requirements, and uniform association with the lesions is believed to indicate causal relationship. The fact that no such lesions have been produced in the few inoculations with *Trichoderma* sp. does not exclude this hypothesis, as the field evidence indicates that the lesions occur only under very unusual conditions, which are not well enough understood to be duplicated in artificial inoculations.

## PESTALOZZIA SPP.

Species of Pestalozzia have occasionally appeared in cultures made by planting damped-off seedlings in prune-agar plates. In some cases the spores had two dark cells, suggesting *P. hartigii*. The occurrence of *Pestalozzia* spp. in cultures from damped-off seedlings has not been sufficiently frequent to indicate parasitism strongly. Some interest, however, attaches to the positive inoculation results reported by Spaulding (*25*). The fact that *P. funerea* is widespread and common on dead coniferous material and that he succeeded in killing 1-month-old seedlings of western yellow pine by inoculation with pure cultures of it indicates that it may be of some importance as a cause of damping-off. His single experiment is the only one which has been noted on seedlings still of damping-off age. Further experiments with it on young pine seedlings are desirable. Its slow growth and rather slow fruiting tendencies would make it rather difficult to demonstrate in diseased seedlings always filled with fast-growing saprophytes, so that failure to obtain it frequently by cultural methods is no proof that it does not occur more or less frequently in the seedlings.

## BOTRYTIS CINEREA

*Botrytis cinerea* (*B. douglasii*, *B. vulgaris*), frequently connected with the damping-off of seedlings of various plants and with needle diseases of the young shoots of coniferous seedlings, does not seem to have been reported as causing damping-off of conifers. *B. cinerea* has never appeared in the

writer's cultures from Colorado and points farther east, but was easily obtained from western yellow-pine seedlings growing in sterilized soil in the plant pathological laboratory at the University of California. The lesions on which it was first obtained occurred just above the soil surface and produced the Botrytis spores directly on the lesions almost as soon as the lesions themselves became evident. The spores and sporophores appeared to be typical of *B. cinerea*.

Inoculations had been made prior to this time with a culture of *Botrytis cinerea* from apples from the Pacific Northwest furnished by Dr. J. S. Cooley, of the Bureau of Plant Industry. The pots inoculated were kept covered with glass plates from the time they were autoclaved until the close of the experiment. Jack pine was used in the test, and the inoculum was probably a mixture of cultures on steamed rice and prune agar (record on this point is lacking). Parallel tests were made on three soil types. On two of these the germination was very poor in all pots, and damping-off was present in the controls as well as in the inoculated pots. In the third soil type, a poor soil from Takoma Park, D. C., in which inoculations of *Pythium debaryamum* had proved unsuccessful, 44 seedlings appeared in the five control pots during the first few days after germination began, while in the five pots of *B. cinerea* but two seedlings appeared in the same time. Additional seedlings appeared later in both, but with still approximately three times as many in the control pots as in the pots inoculated with the fungus. Damping-off was somewhat, but very little, heavier in the Botrytis pots. The experiment indicates germination loss as a result of the presence of *B. cinerea*, but must be repeated to give conclusive results.

Mainly because of the known parasitic ability of *B. cinerea* on seedlings of other plants and on older conifers and the strong indications of parasitism seen in the seedlings at the University of California, rather than on the basis of the single inoculation experiment, it is believed that this fungus will probably be sometimes found causing damping-off of conifers. It is a fast-growing organism on prune agar, and failure to obtain it at most of the nurseries at which cultures have been made is strong evidence that it is not present in the damped-off seedlings at these places. Spaulding's failure to obtain it frequently from the seedlings with which he worked (*26*) is further evidence that in the East and Middle West at least it is not important as a cause of damping-off of conifers.

## MISCELLANEOUS FUNGI

*Alternaria* sp. has been very frequently encountered in cultures from dead coniferous material, both in the tender seedling stage and in older nursery stock. No inoculation tests with it have been made. It is not believed to be important as a damping-off parasite, but its frequent

occurrence, especially on damped-off seedlings under moist chamber conditions, makes a test of its parasitism desirable.

The common large *Mucor* sp. is another fungus frequently found in agar cultures from damped-off pine seedlings. Two strains, one apparently pure and the other of somewhat doubtful purity, have been tested in inoculation on jack pine in autoclaved soil. In experiment 31 (Table II shows results of other fungi in this experiment) there was distinctly less damping-off in the 10 pots inoculated with cultures of *Mucor* sp. than in the 25 control pots. In the experiments on sand treated with acid and lime, the two Mucor cultures gave slight indication of parasitism on jack pine and still less indication on western yellow pine. In both cases the differences between the inoculated and control plots were well within the limits of accidental variation. It seems probable that strains of Mucor are not of any importance as damping-off parasites and that their frequent occurrence in the cultures from seedlings, as in the case of *Trichoderma* sp., can be explained by the fact that *Mucor* sp. is a common soil-inhabiting saprophyte and is able to grow very rapidly in prune agar.

*Penicillium* sp. of the common green type has occurred more or less frequently in cultures from damped-off seedlings. Mixed cultures containing *Penicillium* sp., like the cultures of *Mucor* sp., apparently caused a decrease rather than an increase in damping-off in the 10 pots of jack pine to which they were added in experiment 31, and on both jack and western yellow pine in the experiment on soil treated with acid and lime.

*Aspergillus* sp. of the common type with black spore heads, obtained from damped-off western yellow pine at Washington, D. C., when inoculated on five pots of jack pine in experiment 31, apparently caused a decrease rather than an increase in damping-off. In the experiment on jack and western yellow pine on sand treated with acid and lime, the same was true for this culture, and also in the case of western yellow pine for another strain of Aspergillus taken from damped-off western yellow pine from the Kansas nursery, at which the experiment was conducted. This latter culture had no effect on jack pine. *Aspergillus* sp. has not appeared in culture from seedlings often enough to warrant any suspicion that it is concerned in causing damping-off.

*Phoma betae* (Oud.) Fr. (pure culture furnished by Dr. H. A. Edson) in experiment 31, Table II, had no apparent effect on damping-off of pine. The same held true in western yellow pine in the experiment on sand treated with acid and lime, and while a slight decrease in germination and a slight increase in damping-off was noted in the jack pine inoculated with *P. betae* in this latter experiment, the difference was easily within the limit of accidental variation. The failure of this damping-off parasite of beets to affect pines was to be expected, in view of the specialization of the fungus to beets indicated by the systemic character of the infection it produces on them (*8*).

Two pots of pine in autoclaved soil were heavily inoculated with agar cultures of the species of Phoma which has been shown to cause the blight of young cedar (*10*). No damping-off resulted, although in the same experiment both *Corticium vagum* and *Pythium debaryanum* proved strongly parasitic. It has not been found in cultures from damped-off seedlings.

An unidentified species of Phoma from blighted 2-year-old seedlings of western yellow pine from Montana, used on five pots of jack pine in experiment 31, apparently resulted in decreased rather than increased damping-off.

Inoculation with a bacillus which appeared commonly with *Pythium debaryanum* from damped-off seedlings also appeared to decrease rather than increase damping-off in experiment 31, and in the experiments on soil treated with acid and lime to increase the loss, but in both cases to an extent explainable as accidental variation.

The statement made in several of the foregoing paragraphs that the organisms considered apparently decreased damping-off in experiment 31 is not necessarily paradoxical, in view of the possibility of competition between saprophytic organisms and accidentally introduced parasites.

It is realized that not all of the possible damping-off parasites have been tested. In some cases it has proved very difficult to obtain from damped-off seedlings any of the known parasitic organisms. It is desirable to make further attempts to obtain, and to carry on inoculation experiments with, slow-growing organisms not likely to appear in the planted plate cultures used by the writers for isolation purposes.

## OTHER PARASITIC DISEASES

*Discosia pini* Heald has been found on western yellow pine in the same way as described by Heald (*17*) and at the same nursery. Observations confirm his conclusion that it does little or no harm. Cultures were obtained by making dilution plates of the spores, but the subcultures were not carried long enough to obtain fruits. The same fungus was found occurring in just the same way on white pine from a nursery in Georgia.

Another fungus occurring on living pine seedlings of the damping-off age without causing the decay typical of damping-off is the European rust *Melampsora pinitorqua* (A. de B.) Rostrup. This rather dangerous parasite has not so far been reported authentically from America, and its importation should be very carefully guarded against. It most commonly attacks the young needles and shoots of trees 10 to 30 years old, but it may also produce its orange sori on seedlings which have just appeared above the soil surface (*11*, *24*). Hartig (*11*) has found it attacking as many as two-thirds of the seedlings in a stand of Scotch pine, producing spores on the cotyledons and hypocotyls when the seedlings were only 2 months old. Seedlings recovered whose cotyledons

only were attacked. It is reported on Austrian and Corsican pine, on *Pinus montana*, and even on white pine (*5*). The same or a similar rust has been reported on species of Abies (*24*). The alternate stage occurs on poplars (*Populus tremula, P. alba, P. balsamifera candicans*, etc.), and as it attacks stems as well as leaves, importation of poplar as well as of coniferous nursery stock may introduce the disease.

## WHITESPOT

Light-colored shrunken lesions sometimes appear at the bases of the stems of pine seedlings as a result of excessive heat at the soil surface. The lesions (Pl. B, fig. 4, 5) are characterized by shrinkage, light color, definite limitation, and the fact that such one-sided lesions as occur are usually on the south side of the stem. They have undoubtedly been confused with damping-off in the past. Unlike damping-off, the lesions do not extend longitudinally until some days after their appearance and then apparently only as a result of the invasion of the original lesion by fungi. Upward conduction of water is not interfered with. Losses are occasionally serious, especially where there is little shade or the soil is loose and inclined to become dry at the surface. This type of injury has been described in more detail by Münch (*19, 20*) and Hartley (*15*), who present evidence as to its relation to heat. A case of damping-off recently reported in red pine (*18*) in which the loss was limited to soil containing raw humus allowed to dry at the surface may be an example of confusion between whitespot and parasitic damping-off. Münch emphasizes the likelihood of heat injury on raw humus soils.

## MECHANICAL INJURIES

Mechanical injuries of different types are rather frequent in coniferous seed beds during the first month after germination. This is particularly true of species which have small seed and therefore produce delicate seedlings. Red pine, with a seed of intermediate size, is also very subject to mechanical breakage because of the unusual brittleness of the stems during the first two or three weeks after the seedlings appear above soil. Counts of obviously mechanically injured seedlings were continued throughout the damping-off season in beds of red, jack, and western yellow pine in Nebraska, Kansas, and the Lake States, in connection with seed-bed disinfection tests. A survey of the data on a large number of the untreated plots taken at random shows that the total loss from mechanical causes may vary from 1 to 10 per cent of the entire stand, with losses of 3 to 3.5 per cent common, and below 2 per cent or over 5 per cent rare.

Types of mechanical injury which cause loss in the seed beds are (*1*) the washing out of the germinating seed or of very young seedlings; (2) the actual breakage of the stems by high wind, hail, beating rain, or the feet of birds or animals; (3) the eating or pulling up of the seedlings

by insects, rodents, or birds; (4) the cutting off of the roots below ground by insects in the soil which presumably feed on them, or by moles which accidentally break them in working through the soil; and (5) the breaking over of the stem, usually at the soil surface, as a result of repeated bending by wind or possibly by other agencies, sufficient to finally cause the collapse of the cortex without transverse rupture of the epidermis or vascular system.

The first four types need little mention, as there is no danger of their being confused with any of the other types of disease mentioned in this article. A few observations on the factors influencing the washing out or breakage of seedlings in artificial watering may be of interest. It develops that the fine spray from nozzles which some nurserymen use in watering germinating seed beds will in some cases cause much more loss through the washing out or breakage of seedlings than some other methods of watering. The best types of stationary sprinklers are less harmful. Another much more drastic method of applying water has also been found relatively harmless. It appears that the washing out which commonly occurs is due not so much to the amount of water applied as to the angle at which it strikes the bed. This at least seems to be the case in the sandy soil where most of the writers' watering experiments were conducted. A fairly strong spray from the nozzle, by striking the surface of the bed at an angle, as it practically always does, seems to displace soil particles to a much greater extent than a very much heavier mass of water striking the surface of the bed directly from above. Very shallow-sown seed beds have been watered without injury, by applying the water directly from the end of a 1-inch hose line without nozzle or even coupling, the end of the hose being so held that the water was spread out over the hand of the man holding it and fell vertically on the beds in a rather thin sheet. In this way water was applied to the beds with good pressure from large mains and hose leads at the rate of 15 gallons per minute, while on the same beds a nozzle closed so as to give a spray and delivering only from 3 to 5 gallons per minute caused considerable washing out.

The fifth type of mechanical injury mentioned in the foregoing is one which, though probably not uncommon, is very difficult to distinguish from whitespot due to heat, and may also be confused with damping-off. Plate B, figure 7, shows a seedling which was found broken over with characteristic white-spot symptoms at a time following high wind and cold, cloudy weather. The conditions were such as to seem to preclude the possibility of heat as the cause, and the lesion differed from the ordinary heat lesion in having the constriction more definitely limited to a particular point, not involving the stem either above or below that point. Much the same symptoms were later produced in a seedling of western yellow pine by exposing it to a strong wind. Simple mechanical bending was then tested on seedlings of jack, red, and western yellow pine. As expected from the experience in the seed beds, lesions such as those in

figure 7 could not be readily produced by bending red pine, as the stems broke squarely off, and immediately wilted. In one or two cases, however, breaking over with one-sided lesions was obtained without apparent rupture of the epidermis. In western yellow pine repeated bending, not carried quite far enough to break the stem, in most cases produced a collapse of the cortex at a point on the side away from the application of the bending force, with a resultant weakening of the stem at this point so that a sharp right-angled bend could be made without further injury to the stem. In long repeated bending the entire perimeter became more or less constricted at this point and the seedling lost its ability to stand erect. With jack pine, the species in which the supposed wind injury was observed in the seed beds, similar lesions were more easily produced either by artificial bending or by the application of a strong draft of air from a pressure vent. If the mechanical bending included a slight twisting movement or if the seedling was so placed that it was whipped sideways as well as downward by the blast of air directed against it, the collapse of the cortex at the point of bending could be made even more complete than that observed in the nurseries. The whitening of the stem both above and below the constriction, shown in Plate B, figure 7, was not produced by artificial bending or exposure to air currents to any great extent except in lesions which occurred just at the soil surface. It is believed that the change of the epidermis above and below the point of constriction from red to nearly white, indicating the loss of sap from the cells, came as a result of bruising against the soil rather than as a result of bending alone. The stems of young seedlings are so susceptible to bruising that even stroking the side of the stem with a smooth piece of wood or a rubber-tipped rod results in the development of a lesion characterized by sunken surface and more or less discoloration.

What apparently takes place in these cases is the loss of sap from the cortical tissue, and probably, in view of the loss of red pigment, from the epidermal cells as well. Whether this is a result of an actual rupture of the internal tissues or from an increase in the permeability of the tissue as a result of the mechanical stresses developed is not known. It should be pointed out that there is nothing especially different from the collapse of the cortex under bending and collapse due to external pressure. In both cases the injury is believed to be caused by pressure. This belief is supported by the fact that in the case of experimental bending it is the cells on the side of the stem on the inside of the bend which first collapse, these, of course, being the ones which get the most pressure in the course of the bending. That the vascular tissues are not seriously affected is evidenced by the continued turgor of the leaves. In a seedling with a much more serious and extensive lesion than the one figured, resulting from mechanical bending, the leaves were still turgid 16 days after the injury, and the prostrate stem had shown negative geotropic response,

the cotyledons and the upper part of the stem turning upward away from the soil. The epidermis, while showing no evidence of serious injury beyond the loss of pigment, may suffer microscopic ruptures. On the upper side of a yellow-pine seedling at the point where a lesion was being developed by bending, observation with a hand lens showed that minute drops of liquid were extruded from the epidermis apparently at small ruptures, which, however, could not be detected after the drops had disappeared. Stomata may, of course, have been the points of exit of the sap.

The importance of lesions produced by bending without outright breakage in the seed beds is slight, unless very high winds occur during the first 10 days after the appearance of the seedlings. The fact that the lesions, like white-spot lesions due to heat, remain definitely limited for a week or more, together with their lighter color, serves to distinguish them from those caused by the usual damping-off organisms. Positive differentiation on the basis of color alone is not entirely safe, three or four days' observation being necessary to determine whether or not a lesion is parasitic.

It may be mentioned that in older plants a girdling of Russian wild olive, several months old, by a transverse lesion at the soil surface, followed by death a number of weeks later, and the girdling and local collapse of the soft young shoots of 2- or 3-year-old pine nursery stock, both observed in regions of high wind, may also prove to be due to excessive bending.

## DROUTH INJURY

Death of young seedlings from drouth is undoubtedly sometimes confused with damping-off. Seedlings so young that the stems have not finished elongating may remain erect for some time after being killed either by drouth or normal damping-off, as the compactness of the tissues apparently prevents immediate collapse from water loss, and decay takes place but slowly. When the seedlings are old enough to have stiff, wiry stems, difficulty again arises in distinguishing between seedlings killed by drouth and those killed by the late type of damping-off. Therefore during the first week and after the third week from germination it is not usually possible to distinguish drouth injury from damping-off by the condition of the individual seedling. Fortunately the distribution of the diseased seedlings often gives a clue. Damped-off seedlings, especially in the late damping-off type, are usually so grouped as to definitely indicate infection foci. This is not the case in drouth injury.

At the intermediate age of one to three weeks it is often possible to distinguish between drouth injury and damping-off by the condition of affected seedlings. At this stage seedlings dying from drouth show prompt and unmistakable wilting, while the damped-off seedling at first remains turgid, typically falling on account of the decay of the base of the stem before wilting of the rest of the stem takes place. Seedlings just

fallen over as a result of water shortage therefore exhibit uniform bending of the entire stem, while seedlings fallen from damping-off or from white-spot usually have a rather sharp bend in the lower part of the stem, and little or none in the upper part.

In a number of western commercial nurseries in which no provision is made for artificial watering drouth injury is in certain seasons a serious matter. During a normal season and in a loamy soil loss from drouth in first-year seed beds is probably not a serious matter east of the Mississippi River. In nurseries, first-year seedlings more than a month old apparently suffer less often from drouth than 2- or 3-year-old seedlings as they have their roots well established, but are not large enough to crowd each other and rapidly exhaust the soil moisture.

Soft-stemmed seedlings so far injured by drouth as to be badly wilted may still recover if supplied with water within a few hours after wilting.

## SUMMARY

(1) Damping-off is the most serious disease of very young seedling conifers. A number of symptomatic types are described, part of which are figured in Plate B.

(2) *Corticium vagum*, *Pythium debaryanum*, and other oomycetes; *Fusarium moniliforme*, *F. ventricosum*, *F. solani*, and other species of Fusarium; *Trichoderma* spp.; and *Botrytis cinerea* have been isolated from damped-off conifers, and are believed to be able to cause the disease. Spaulding's work indicates that *Pestalozzia funerea* can also cause damping-off of pine. In artificial inoculations on pines in autoclaved soil, the first three species named proved to be especially virulent parasites. All except *Trichoderma* spp. have given more or less indication of parasitic ability in inoculation experiments.

(3) For *Corticium vagum* 12 coniferous hosts are listed (p. 531). One strain was maintained in artificial culture continuously for eight years without perceptible loss of virulence. There is a marked difference in virulence between different strains, which bears little or no relation to the host from which the strain was isolated. For example, strains from spruce and sugar beet, respectively, proved more virulent in inoculations on pine seedlings than did any of the strains originally isolated from pine. There was furthermore no indication that passage through seedlings and reisolation resulted in any increase in virulence. *C. vagum* was found especially virulent in inoculations on a very sandy soil treated with sulphuric acid, followed by lime. Slight virulence was indicated in preliminary inoculations on untreated soils.

(4) With the possible exception of *Pythium debaryanum*, *Corticium vagum* appears to be the most important single damping-off parasite on conifers, certain species of Fusarium also being probably important, and the remainder of the organisms mentioned unimportant. The available

data do not justify a final statement on the relative importance of the different parasites.

(5) The following fungi have indicated inability to cause damping-off of pines: *Aspergillus* sp. (the ordinary black type); *Penicillium* sp. (the ordinary green type); a rapidly growing species of Mucor from damped-off seedlings and seed-bed soil; *Trichothecium roseum; Fusarium acuminatum; Rosellinia* sp. from nursery soil; *Chaetomium* sp. from maple roots; *Phoma betae;* the species of Phoma which causes red-cedar blight; and a third species of Phoma from pine in Montana.

(6) At least with some of the fungi found parasitic, heavy inoculation and heated soil so favor parasitism that past experiments, mostly conducted under such conditions, do not constitute an entirely reliable basis for deciding what goes on in the seed beds under more natural conditions.

(7) Inoculations in autoclaved soil with certain saprophytic or weakly parasitic fungi have apparently resulted in a decrease rather than an increase in damping-off in some cases.

(8) *Corticium vagum* and *Pythium debaryanum* cause a large part of the damage they do by killing seed or seedlings before they appear above soil. Strains of Fusarium are less inclined to do this. Such losses are often wrongly attributed to poor seed. Some of the damping-off fungi are able to continue to kill the roots of seedlings after they develop rigid stems, so that they do not fall over. This type of trouble is sometimes confused with drouth.

(9) Excessive heat, drouth, or bending may each cause injury closely simulating damping-off. On careful examination these nonparasitic types of injury can usually be distinguished from damping-off by characters described in the last pages of this paper.

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## PLATE B

1. Normal damping-off on western yellow pine; caused usually by *Corticium vagum*, *Fusarium* spp., or *Pythium debaryanum*. Natural size.

2, 3. Blacktop damping-off on jack pine; probably caused by *Trichoderma* sp. × 2. —— = location of surface of soil.

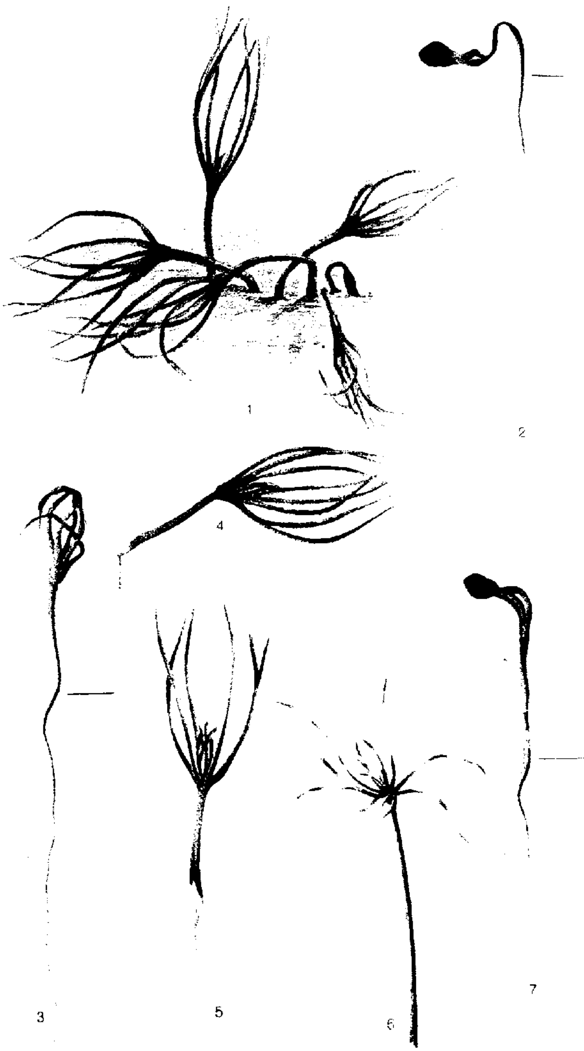
4. Whitespot injury, common type, on western yellow pine; usually due to excessive heat at soil surface. Natural size.

5. Whitespot lesion, one-sided type, on western yellow pine; due to heat. Natural size.

6. Late damping-off resulting from inoculation with *Pythium debaryanum* on red pine more than 5 weeks old. × 1½. —— = location of surface of soil.

7. Wind injury to jack pine. This seedling had fallen over and was propped up for drawing purposes. × 2.

Drawn by Maybell S. Hartley.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# HISTOLOGICAL STUDIES ON POTATO LEAFROLL [1]

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COOPERATIVE INVESTIGATIONS BETWEEN THE BUREAU OF PLANT INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE AND THE AGRICULTURAL EXPERIMENT STATION AT CORNELL UNIVERSITY

## INTRODUCTION

External symptoms are often insufficient and sometimes misleading in the identification of certain plant diseases, and the pathologist must resort to the studies of internal morphology and physiological reaction to bring otherwise problematical questions to a satisfactory conclusion. The necessity of such an attitude is most clearly exemplified in the case of the Irish potato leafroll diseases, which have been the subject of much discussion and controversy for the last decade, both in this country and in Europe.

The literature of potato leafroll is voluminous and varied. The most important articles, about 600, were reviewed by Appel and Schlumm-berger (*1*)[2] in 1911, and about three years later this list was extended by Orton (*4*), in which he reviews the history of this disease and related troubles and the theories regarding its cause.

The ready identification of leafroll in the field and its separation from other maladies is difficult, and there is much confusion over diagnosis. Our knowledge of the pathological physiology of this disease is incomplete, and an understanding of the histological changes in the diseased tissues is only in its beginnings. Very little is known in regard to the chemical changes which accompany the disorganization processes in the cells and tissues, and nothing definite is known about the origin and progress of the disorganization which sometimes leads to a complete obliteration of the walls and contents of the elements of the conducting system for plastic materials.

The pioneer researches of Quanjer (*5*) have given an impetus to new investigations along the lines just mentioned and have diverted our views from the fungus culture to the histological and physiological side of the problem.

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[1] This work was begun in the field laboratory of the Office of Cotton, Truck, and Forage Crop Disease Investigations at Greeley, Colo., in the summer of 1916, and was continued in the Department of Plant Pathology at Cornell University under the direction of Prof. H. H. Whetzel and Dr. H. A. Edson, to whom the writer wishes to express his gratitude for their courtesy and helpful suggestions. To Dr. A. J. Eames, of the Department of Botany at Cornell University, the writer is especially indebted for the constant advice and criticism received in preparation of materials, interpretation of slides, and editing of the paper.

[2] Reference is made by number (italic) to "Literature cited," p. 570.

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While taxonomic, inheritance, and control studies are being carried on at present by various investigators, the following studies have for their purpose the testing of Quanjer's hypothesis with both European and American material, and the adding of new facts to the already existing knowledge. A former paper (2) on the anatomy and development of the normal Irish potato plant furnished a background for the pathological study and facilitates a more accurate interpretation of the existing conditions.

It is realized that an adequate inquiry into the problem of potato leafroll from the viewpoint of the morphologist should first of all comprise a detailed seasonal study of the normal plant grown under different ecological conditions, with the purpose of correlating the influence of external factors with changes in internal anatomy. These investigations should then be extended to a study of plants affected with diseases of parasitic and physiologic origin, to determine in how far the delicate internal structures respond to the stimulating effect of parasites and their toxins, as well as to unfavorable conditions of growth.

A detailed microscopical study extending over several generations should make it possible to trace the development of the disease from the first cell response through the various changes, culminating in the cessation of function and death; while microchemical investigations should afford an insight into the physiological activities of the diseased plants, especially in respect to assimilation and translocation. A critical study of the data obtained should either emphasize or discredit the value of comparative anatomical studies for diagnostic purposes, and might permit a grouping or separation of diseases on the basis of pathological anatomy according as the symptoms found were similar or unlike.

Present circumstances make it necessary for the writer to interrupt studies of this character for an indefinite period while they are still incomplete and preliminary, but sufficient progress has been made on a few of the general questions of foremost interest to justify their presentation in the hope that they may be of interest and value to other investigators. This paper will be limited to the presentation of a comparative study of certain European and American types of leafroll, together with observations on the progressive histological changes in the tissues.

## EXPERIMENTAL MATERIALS AND METHODS

Tubers for the study of European leafroll were obtained from Dr. Quanjer, Wageningen, Holland, and from the Agricultural College, Copenhagen, Denmark. The material representing American leafroll was obtained from the Government station in Greeley, Colo., and from various places in the State of New York.

The methods of killing, fixing, embedding, and staining the material were the same as those used (2) for the normal Irish potato (*Solanum tuberosum*).

## DESCRIPTION OF EUROPEAN LEAFROLL

### LEAFROLL IN PAUL KRUGER VARIETY

Tubers from both normal and diseased potato plants were grown in the disease garden of the Department of Plant Pathology of Cornell University. The plants from the diseased tubers grow more slowly than the normal ones, show a shortening of the internodes of the stem (Pl. 35, A, B) and a pronounced development of axillary shoots later in the season (Pl. 37). The lower leaves, and later the upper ones, become rigid, and the leaflets fold more or less along the midrib. The whole leaf shows the effect of the rolling, and exhibits a spiral tendency, causing the mature plants to stand out prominently among normal individuals. The color of the foliage changes with the progress of the other symptoms. At first the lower leaves show a pale discoloration; in the beginning this is confined to the tip and the margin of the leaflets, gradually spreading and changing to a reddish tinge. At the time of maturity of the plant the tissue of many of the discolored areas is dead and of a brown color. The tubers set normally, but on rather short stolons; the yield is small both as regards number and size. The seed piece is found unexhausted in the soil.

Cross sections through midrib and lamina of a leaf of such a plant show, on the whole, normal histological structures. The fibers, however, are more plentiful, and high magnification reveals slight changes in the peripheral region of the vascular tissue. Here and there a few cells of the pericycle are found to be filled with a deposit, sometimes crystalline, sometimes of globular masses, yellow in unstained sections. When stained with Haidenhein's hematoxylin and safranin, the deposit stains reddish or remains yellow. Mineral acids, either dilute or concentrated, do not dissolve this precipitate; alkalies increase the intensity of the discoloration. Treatment with phloroglucin and hydrochloric acid to test for lignification gives negative results; alcanin, however, produces a reddish color suggesting the presence of cutin or cutin-like substances (Sudan III and Scharlach R, the most typical reagents for cutin are unavailable at present). In other places intercellular spaces have formed between the cells of the phloem. These cavities are filled with a secretion rather plastic in nature and reacting similarly to the treatment of chemicals as does the crystalline deposit found inside the cells just mentioned. The cells of the pericycle in such a region are radially elongated and slightly distorted.

Sections through the lamina show a disorganized condition both of palisade tissue and of spongy parenchyma in those regions which show external symptoms of the disease most strikingly. The cell wall, though not thickened, has undergone chemical changes, having become cutinized. The lumina of these cells are filled with a precipitate of the nature described for the diseased cells of the vascular tissue of the midrib. The

petiole, like the midrib, also shows normal conditions as regards the amount and arrangement of vascular tissue. The fibers are more numerous and of a larger diameter than those found in normal sections. The secondary walls of these elements are often very thin and only very slightly lignified. The smaller type of fiber, especially numerous in the inner phloem, has its secondary walls heavily lignified, and the lumina are sometimes filled with the granular deposit. Intercellular secretions are evident in the peripheral region of the outer phloem and in some of the cells of the pericycle. The inner phloem is, on the whole, normal. Plate 39, A, shows a disorganized phloem group with the wheel-shaped arrangement of the parenchyma cells surrounding it.

The diseased condition becomes more pronounced in the growing region and especially in the nodal region of the upper part of the stem. Cross sections through the upper three internodes show nearly all types of pathological changes observed in this study. Plate 38, C, and Plate C, figures 1 and 6, illustrate the formation of intercellular cavities between the sieve tubes of an inner phloem group. The primary wall separates—a fact not easily observed in unstained material—and the space resulting from the separation of these cells is filled with a yellow substance, gummy and plastic in nature. Similar changes occur in the outer phloem (Pl. 38, B, and Pl. C, fig. 2). In more advanced stages the formation of intercellular spaces has progressed to the extent of causing bending in and crushing of the phloem cells. In the final stage of disorganization the phloem cells are almost always filled with a granular substance, while the walls themselves have become cutinized or undergone similar changes (Pl. C, fig. 4). Lignification of either cell wall or cell content has never been observed.

The diseased condition is most severe in the nodal region of the upper stem, and it is mostly the outer phloem that is affected. However, cells of tissues other than the phloem exhibit pathological changes. Plate 38, A, and Plate C, figure 3, show the effect of the disease in the cells of the cortex and pericycle. The large cortical cells adjacent to the fibers are filled with the granular precipitate, the walls themselves having become cutinized, and the small triangular intercellular spaces are filled with the yellow secretion. Intercellular spaces are seen to be forming between the cells of the pericycle; they originate among the outer fibers and gradually advance toward the cambium. The cells of the pericycle have become radially stretched and at the same time have increased in number, forming a broad sheet of tissue between the fibers and the primary phloem (Pl. 42, A). In normal sections the primary phloem groups, if not directly adjacent to the fibers, are almost always separated by only one or two layers of cells.

A different type of pathological condition is sometimes observed in both stem and leaf sections. Plate 39, B, shows a section taken from the internodal region of the middle part of the stem. Only the cells of the

inner phloem are affected. The individual groups are normal in size; there are a few cell wall thickenings, but no evidence of formation of intercellular spaces. Most of the walls of the sieve tubes and companion cells have become faint and in places are completely obliterated. The cell content is mostly disorganized protoplasm. These diseased groups take the cellulose stain, less distinct, however, than the normal cells.

The basal region of the stem and the underground parts are normal in structure; the flowering axis has not been examined.

### LEAFROLL IN MAGNUM BONUM VARIETY

The tubers were obtained from the Agricultural College, Copenhagen, Denmark, and planted, together with other varieties under investigation, in the disease garden of the Plant Pathology Department of Cornell University. The plants grew normally at first; most of them, however, developed signs of disease in late summer, though a few showed symptoms of leafroll very early. Material for study was taken in late July from a plant which was large and well developed, but the axillary shoots of which were erect and broomlike in appearance. The lower leaves were normal, the intermediate and upper ones reduced in size and folded along the midrib. Discoloration of the leaves was only slight.

Microscopic examination of both stained and fresh material shows that the vascular tissue of the midrib is abnormal in quantity with thickenings of the walls of the phloem adjacent the fibers (Pl. 45, A). These thickenings stain black with Haidenhein's hematoxylin—that is, they are of cellulose. The lamina of the leaf, however, is severely diseased. The partly obliterated cells are filled with a granular or globular deposit, which is yellow in unstained sections and of the same nature as found in the diseased cells of the phloem in the Paul Kruger variety. The petiole shows also normal anatomical structures. The bundles of the petiolar wings are above normal in size. Fibers in both external and internal region are of unusually large number (Pl. 45, B).

Stem sections, whether taken near the distal or the basal end, show normal structures. The fibers here too are very plentiful. The external phloem shows a large amount of secondary sieve tubes and medullary ray tissue, all of which appears normal and functional.

### LEAFROLL IN EARLY ROSE VARIETY

The tubers were obtained from the same source as were those of the Magnum Bonum variety; they were planted at the same time and received the same cultivation and care. The plants grew very unevenly. A few of them attained normal size, but most of them remained small, showing symptoms of severe leafroll. Plate 36 shows such a plant. The stem is not above 15 cm. tall; the leaves are rolling, but are not reduced in size. There is slight, indistinct discoloration of the lamina.

The general amount and arrangement of the vascular tissue of the midrib is normal. Fibers are wanting, and the phloem groups of the inner

region show disorganization of cell content and partial obliteration of the walls of the individual cells. The spongy parenchyma of the lamina is abnormal in places; the cells in such a region are filled with a yellow deposit and the walls themselves are of a yellow color. The petiole is normally developed. Fibers are present, and the phloem cells in the region of the inner fibers are more or less thickened.

Stem sections are, in general, normal. While the outer phloem shows no evidence of pathological condition, the cell walls of the inner phloem groups are partly destroyed and the protoplast disorganized. In no instance, however, is there a thickening or discoloration of the walls of these cells.

A summary of the results of microscopic study on European leafroll shows the following conditions:

1. Plants with symptoms of typical leafroll always show pathological changes in the vascular tissue.

2. These pathological changes are the more pronounced the earlier and more intense the external symptoms appear.

3. While the primary phloem groups are most commonly affected, the cells of the pericycle and fibers as well as cortex are often found diseased.

4. The phloem groups are shrunken and completely destroyed only in rare cases. Most usually the cells of the groups are separated by intercellular spaces; the cells may later be crushed and the cell content changed into a granular or globular, gummy deposit. There is seldom a complete destruction of the individual cells in a group and never a sign of lignification.

5. The number of primary phloem groups destroyed is not always very extensive, and since the development of secondary elements is very pronounced the translocation of elaborated food material from the leaves to the tubers is not prevented or only slightly interfered with.

## DESCRIPTION OF AMERICAN LEAFROLL ON COLORADO MATERIAL

The cause of the falling off in yield of the Irish-potato crop in northern Colorado in 1911 was thought by local growers to be species of Fusarium and Rhizoctonia, but others have believed that, though there is a variation in the external symptoms observed, the Colorado disease is the type of leafroll described by Appel and other investigators in Europe.

The material for study was obtained from the Government station at Greeley, Colo., and included one seedling and two standard varieties: Netted Gem and Pearl. These plants were under observation throughout the summer, the progress of the symptoms being recorded at definite intervals.

### LEAFROLL IN POTATO SEEDLING

The mature plant was dwarfed, erect and broomlike in appearance. The leaves were reduced in size and folded characteristically along the midrib. The seed piece was found unexhausted in the soil.

Cross sections through the petiole show normal structures. A few cells of the inner phloem have walls thickened adjacent to the fibers. The cells of the epidermis, though normal in structure, are filled with a dirty-gray granular precipitate. The stem tip, especially in the nodal region, shows a few shrunken inner phloem groups. The cells of the pericycle are slightly stretched radially; a few of the cells have become separated and the intercellular spaces formed are filled with the yellow secretion. Other cells in the same region, but including the cortex, are filled with the granular precipitate (Pl. 41, B) characteristic of the diseased tissue in the plants of the Paul Kruger variety (Pl. 38, A). The symptoms become less pronounced lower down the stem; yet here and there completely disorganized phloem groups are seen, especially in the inner region.

### EFFECT OF LEAFROLL NO. 15000 ON AN UNKNOWN VARIETY

This variety had been selected the previous year (1915) for suspected leafroll. The plants developed normally and attained a large size, becoming spreading later in the season. Symptoms suggestive of leafroll appeared only late in the season. The leaves rolled in a funnel-shaped fashion and showed a slight discoloration of the lamina.

A microscopic examination showed only slight pathological changes in the distal region of the stem. A few intercellular spaces were noticed between the cells of the outer phloem, and these cavities were filled with the typical yellow excretion.

### LEAFROLL OF THE NETTED GEM VARIETY

At least 40 per cent of the plants grown in one of the experimental plots of the plant-breeding station at Greeley showed a characteristic abnormal rolling of the leaves, and from these plants tubers were selected in the fall of 1916 to be grown the following winter in the greenhouse of the Plant Pathology Department of Cornell University. The plants developed normally at first; later the leaves turned yellow and became dry in texture. Nearly all of the leaves showed symptoms of rolling, but unlike that observed in typical leafroll.

Cross sections through petiole show pathological changes in outer phloem and pericycle. In a few places the cells have become completely obliterated, resulting in the formation of extensive cavities. Other disease cells are filled with the granular precipitate (Pl. 41, A), in which are sometimes found embedded starch grains. Sections through the median portion of the stem show all gradations of the disease. Large intercellular spaces are found in the pericycle, and these often extend to the cambium. The fibers of the outer phloem frequently contain the granular deposit also.

A summary of the results of the study of types of leafroll from Colorado shows the following conditions:

1. The pathological changes, though less intense, are of the nature observed in the European disease.

2. Plants developing external symptoms of disease late in the season show normal histological structure of the vascular system.

3. The internal changes were most pronounced in the Netted Gem variety. Although the tubers came from stock having the appearance of leafroll, the plants grown from them did not exhibit external symptoms of typical leafroll.

## DESCRIPTION OF AMERICAN LEAFROLL ON NEW YORK MATERIAL

The material was collected by the writer in the fall of 1917 on the occasion of an inspection trip through Ontario County, N. Y. The disease was quite prevalent; in a few instances as many as 30 per cent of the plants were found to be diseased. Only mature plants which exhibited characteristic symptoms of leafroll were selected for study. The material was put into fixing fluid at once and later dehydrated and embedded in the laboratory in the usual way.

### LEAFROLL IN THE NEW YORK RURAL VARIETY

The material was collected in a field at Phelps, N. Y. The plant selected was fairly large, erect, and bushy in appearance. The leaves were slightly reduced in size and showed extreme rolling and reddish discoloration along the margins of the leaflets.

A microscopic examination showed, on the whole, normal development of the vascular tissue of midrib and petiole. In the outer phloem of the midrib a few abnormalities in the nature of intercellular spaces and stretching of the cells of the pericycle were found. In the petiole there were diseased areas in cortex and pith; the affected cells were filled with the granular deposit.

Sections through the growing region of the stem showed pathological changes of great intensity. Just below the insertion of the leaf in that region large lysigenous cavities have formed which extend from the cortex through the vascular ring into the pith. Longitudinally these diseased areas extend into the petiole of the leaf as well as into the upper part of the stem (Pl. 42, B, and 43, B). The peripheral phloem cells show thickenings of the walls; these thickenings are cellulose in nature. A few of the cells of the outer phloem are filled with the granular precipitate.

Median stem sections show a well-developed, normal external phloem; however the internal phloem groups are almost completely destroyed (Pl. 44, A, B). In unstained sections these diseased groups appear as small, yellow, shrunken masses from which the parenchyma cells of the perimedullary zone radiate in a typical manner. High magnification shows that these groups are either the remains of shrunken and diseased primary phloem elements, large intercellular spaces filled with the

yellow secretion, or a combination of these. Plate C, figure 5, shows such a group. We notice that most of the cells of the phloem are more or less collapsed and filled with a granular precipitate; between other cells in the group intercellular cavities have been formed, the cells themselves having remained normal.

The basal region of the stem is normal, with an occasional disturbance in the inner phloem.

### LEAFROLL IN THE DOOLEY VARIETY, MATERIAL A

The material was obtained from a field near Seneca Castle, Ontario County. The plant selected for study was dwarfed, spindly, erect, and broomlike. The leaves were slightly reduced in size and folded along the midrib. Discoloration of the foliage is only noticeable along the margin of the leaflets.

Sections through midrib show normal structures; a few of the groups of the inner phloem have cells with walls and content disorganized. The petiole is severely diseased (Pl. 43, A) in that almost all of the external and most of the internal phloem is necrotic. The vascular tissue of the petiolar wings is abnormally developed; the large amount of phloem in these groups is probably formed as a consequence of the destruction of the phloem of the lateral and median bundles. Sections through the basal region of the petiole show a decrease in the severity of the symptoms; there are a few diseased areas, especially in the outer region extending from the fibers in the peripheral and radial direction (Pl. 40, A, B).

Upper stem sections show the symptoms described for the basal part of the petiole, perhaps less extreme. There are a few phloem groups completely destroyed; and in such a region the cells of the pericycle are always radially stretched. A few cells of the cortex are also diseased and filled with the typical granular deposit.

### LEAFROLL IN THE DOOLEY VARIETY, MATERIAL B

Material B was obtained in the same field. The plants showed symptoms of severe leafroll; reduction in size, broomlike appearance, rolling, and pronounced reddish discoloration of the foliage.

Microscopic sections through the upper nodal region (midrib and petiole were not examined) show normal structures except for one large diseased area which extends from the cortex to the xylem, involving some of the elements of the latter tissue. Other stem sections through both nodal and internodal region show normal structures.

### LEAFROLL IN THE NEW YORK RURAL VARIETY

This material was obtained from another field in Ontario County. The plants exhibited the symptoms described for the above variety, but microscopic examinations failed to show extensive abnormalities. A few stem sections showed diseased cells in the cortex which were filled with the granular precipitate.

A summary of the results of the study of types of New York State leafroll shows the following conditions:

1. There is not always a definite correlation between external symptoms and internal changes. Typical leafroll plants may not always exhibit pathological changes in the tissues.
2. The type of necrosis is that described for European leafroll.
3. In case of severe attack, American varieties showed a more striking pathological condition than did the European varieties under observation.

## CONCLUSIONS

The anatomical studies of both European and American leafroll have failed so far to show a distinct correlation with the external symptoms exhibited by the plant. It is true that in case of severe attack, recognizable by external symptoms as such, we get the same pathological condition; but there are exceptions which do not permit a wide generalization. Typical leafroll plants which early show external symptoms often fail to show extensive necrotic conditions, while plants affected with trouble apparently other than leafroll, exhibit severe pathological changes in phloem and cortex. The writer, however, is fully aware that only one generation of plants has been studied and consequently he is not in a position to give full assurance that he was dealing always with hereditary leafroll. Should a study of the progeny of these plants establish the heredity nature of the disease as well as the constancy in the symptoms, it might then be possible to separate or unite the various types of leafroll on the basis of these symptoms.

There is, however, some reason to suspect that the development of necrotic tissues is not confined to plants affected with leafroll, but that it is common to the so-called degeneration troubles in general, and perhaps to others also.

The pathological changes are most striking in the distal, growing region of the stem. In sections taken farther down and especially at the basal region, signs of pathological changes are less frequent and often not found at all. An acropetal advance of internal symptoms, as claimed by Quanjer (*6*) for "secondary diseased" plants, has not been observed in this study. Similar observations have been made by Schander and Tiesenhausen (*7*) who report the earliest and most frequent occurrence of phloem necrosis in the upper part of the stem and especially in the region just below the insertion of the leaves.

The primary phloem groups are probably the first to suffer the effects of the disease. The first symptoms, as far as this study is able to show, consist of the separation of the primary walls of the phloem cells of an individual group, resulting in the formation of intercellular spaces which are filled with a secretion which is usually yellow in unstained sections. In advanced stages the primary wall of the cells, bordering these cavities, is also discolored. The size and extent of these spaces may increase to

such a degree as to cause a collapse and folding in of the walls of the phloem cells. Often, it appears, the formation of intercellular spaces, though initial, is only an accompanying phenomenon; the pathological changes in the cells of the phloem themselves proceeding so rapidly as to cause a rapid cessation in function and final death. The diseased cells become filled with a granular or globular deposit, probably metamorphosed protoplasm; the cell walls themselves also undergo changes. The deposit is resistant to acids, becomes bright yellow on treatment with alkalis, and gives reaction for cutin; in no instance, however, could the presence of lignin be shown, a fact already claimed by Schander and Tiesenhausen (7), but disputed by Quanjer. It is of interest to note that usually not all cells in a group become affected and even in very extreme cases there are normal, functional cells within a diseased group. The cells which thus remain normal are either parenchyma cells or sieve tubes.

Accompanying the destruction of the phloem groups and the formation of intercellular spaces are changes in the parenchyma cells of the pericycle and perimedullary zone consisting of a radial stretching of these elements and sometimes in an increase in the actual number. Occasionally the pericycle increases to such an extent as to form a band of tissue between the outer fibers and the primary phloem.

An unusual type of phloem necrosis is sometimes observed in plants affected with leafroll. The cells of the phloem are neither shrunken nor chemically changed. The walls, however, are very attenuate, in places completely obliterated, or sometimes slightly swollen (Pl. 39, B). The protoplasm is abundant and usually disorganized. Whether this condition is a step in the progress of the disease and is later followed by cutinization or other chemical changes, the writer can not state. It does not appear to be such though, since this condition is often found in fully mature plants.

The formation of intercellular spaces is not always the first disease phenomenon. In the internal region, near the fibers, a thickening of the walls of both phloem and pith cells occurs. No chemical changes have been demonstrated to accompany these swellings, and the walls remain pure cellulose. Plate 38, D, shows the beginning of cell wall thickening in a primary phloem group. Although this thickening is sometimes very extensive, the cells themselves do not seem to be harmed thereby.

Necrosis, as we have seen, is restricted to certain areas ("*differenzierete Nekrose*" of Küster; *3, p. 309*), notwithstanding the close anastomosing of the individual groups of the phloem and the direct connection of external and internal phloem through the leaf gaps. If a specific virus is the cause of potato leafroll, one would expect all phloem groups to be indiscriminately affected. Very often, however, only certain groups are diseased, and if the attack is severe the pathological condition may still be restricted either to the internal phloem or the external

phloem alone. This condition might be explained by assuming that the individual cells react differently toward the stimulus and that we have a more or less complete antagonism in those groups of cells which suffer only slightly or not at all. If, however, the toxin is localized or the virus is produced *in situ*, differential necrosis of the phloem as well as necrosis of certain cells of the cortex and pericycle becomes explainable.

The accumulation of starch in the diseased leaves, together with the reddish discoloration suggests, of course, inhibition in the process of translocation. This nonremoval of synthesized food material may be caused by lack of minerals or by the partial or complete stoppage of the path of translocation for these substances. In many instances necrosis of the phloem could in itself account for inhibition in translocation even if the phloem of petiole and midrib is mostly normal. In such cases the phloem of the stem, near the point of the insertion of the leaf, is usually severely diseased, much more so than in any other region of the stem. The nonremoval of starch from leaves where necrosis of the phloem is no factor of importance has still to be explained.

The rolling of the leaves and the characteristic xerophytic appearance of the diseased plants is the resultant of many interrelated changes and processes; such changes could not be produced by simple anatomical disturbances; nor can the results be explained on merely a mechanical basis.

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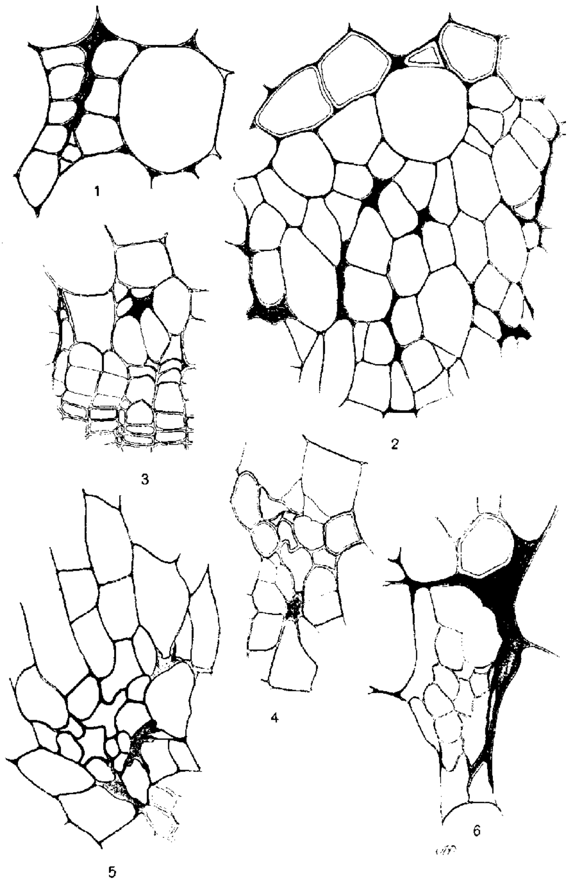


A. Hoen & Co. Lith.

## PLATE C

Camera-lucida drawing of diseased tissues of the Irish potato:

1.—Tranverse section of an internal phloem group showing initial stage in formation of intercellular spaces. (Compare Plate 38, C.)

2.—Tranverse section of external phloem and pericycle showing the same condition.

3.—Transverse section of interfascicular region of mature stem showing necrosis in xylem, cambium, medullary ray cells, and cortex.

4.—Transverse section of external phloem. The cells of the phloem are filled with a granular deposit, yellow in color. Below these cells is seen a large intercellular space filled with a secretion plastic in nature.

5.—Transverse section of internal phloem of mature stem, showing severe necrosis. Note that a few cells in a group are not diseased. (Compare Pl. 44, A, B.)

6.—Transverse section of internal phloem group, showing formation of a large intercellular cavity extending up to the protoxylem and involving a few cells of the phloem. The protoxylem cell above is filled with the yellow deposit.

PLATE 35

A.—Normal potato plant, Paul Kruger variety, Ithaca, N. Y., March, 1916.

B.—Potato leafroll in Paul Kruger variety. Note especially shape of basal leaves and reduction in the length of the internodes. Ithaca, N. Y., March, 1916.


A
B


8

PLATE 36

Typical potato leafroll in Early Rose variety. Ithaca, N. Y., July 30, 1917.

PLATE 37

Typical potato leafroll in Paul Kruger variety. The plant to the left in the photograph is normal, the one to the right diseased. Ithaca, N. Y., July, 1917.


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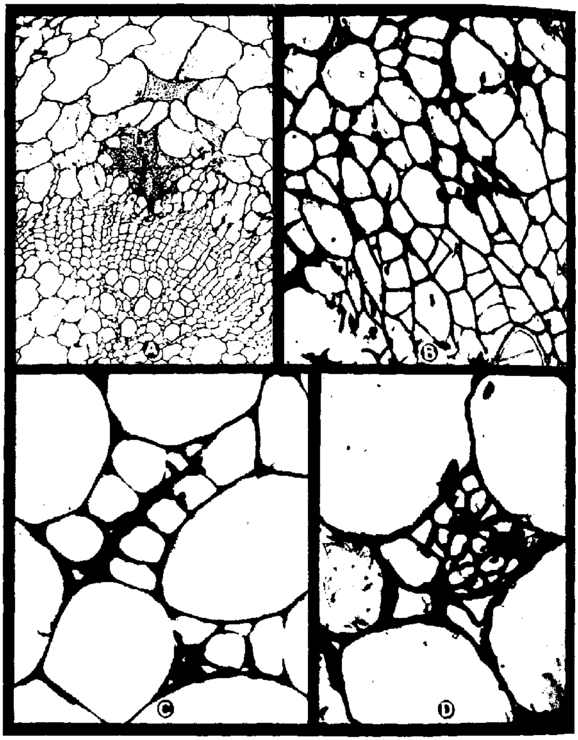

A
B
C
D

## PLATE 38

A.—Cross section through upper part of stem, showing necrosis in outer phloem and cortex. x 104.

B.—Cross section through distal region of stem, showing formation of intercellular spaces in outer phloem and cortex. x 400.

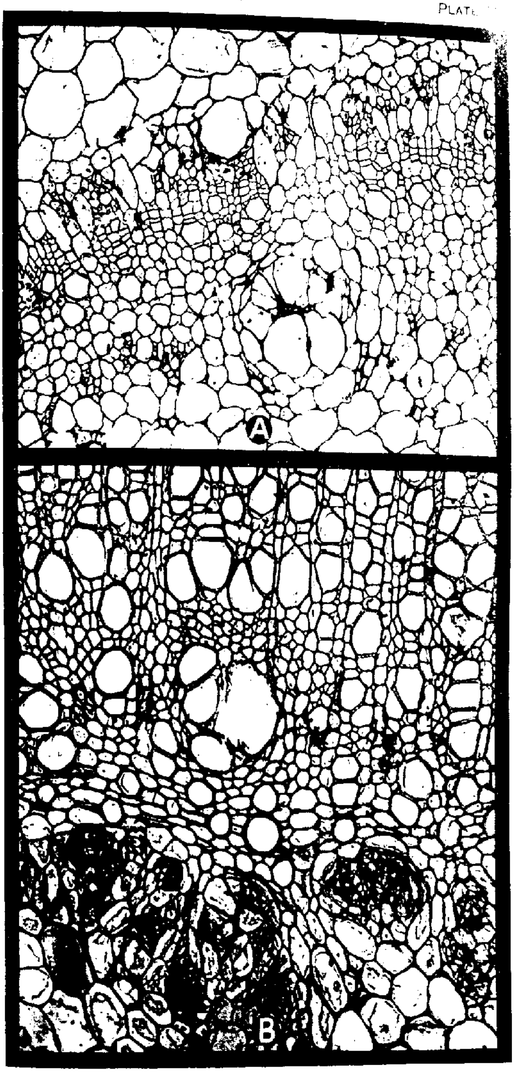
C.—Cross section through distal region of stem, showing formation of intercellular spaces between the cells of the inner phloem. x 800.

D.—View of another phloem group showing cell wall thickening. x 320.

## PLATE 39

A.—Cross section through lateral bundle of petiole showing a diseased phloem group in the internal region and the effect of necrosis on the surrounding parenchyma. × 130.

B.—Cross section of stem showing an unusual type of necrosis. × 130.


A
B


A
B

PLATE 40

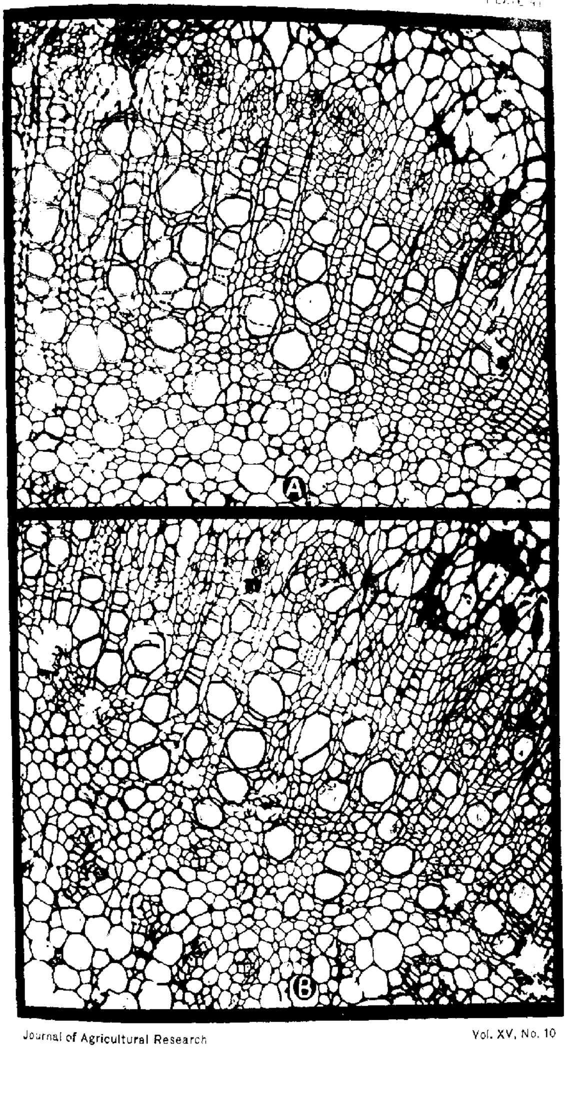
A.—Cross section of stem showing granular deposit in cell of cortex and formation of intercellular spaces proceeding from the region of the fibers centripetally. × 450.

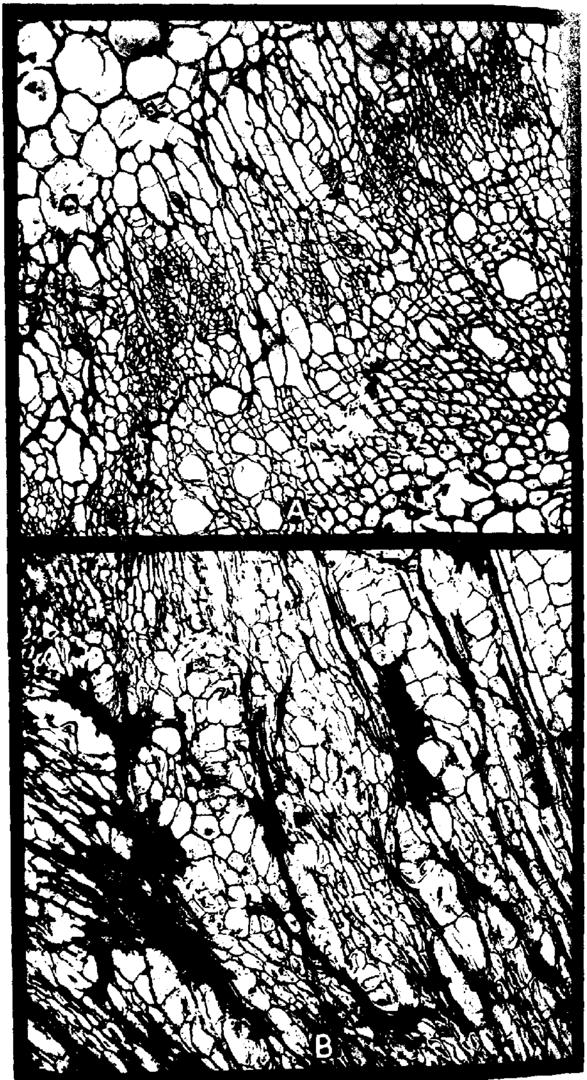
B.—Cross section of stem showing large schizogenous cavity between cells of pericycle and cortex and centripetal advance of the formation of intercellular cavities. × 450.

## PLATE 41

A.—Section of middle portion of stem showing necrosis in cells of cortex and primary phloem. × 130.

B.—Cross section of middle portion of stem showing diseased areas in pericycle and outer phloem. × 130.


A
B


A
B

## PLATE 42

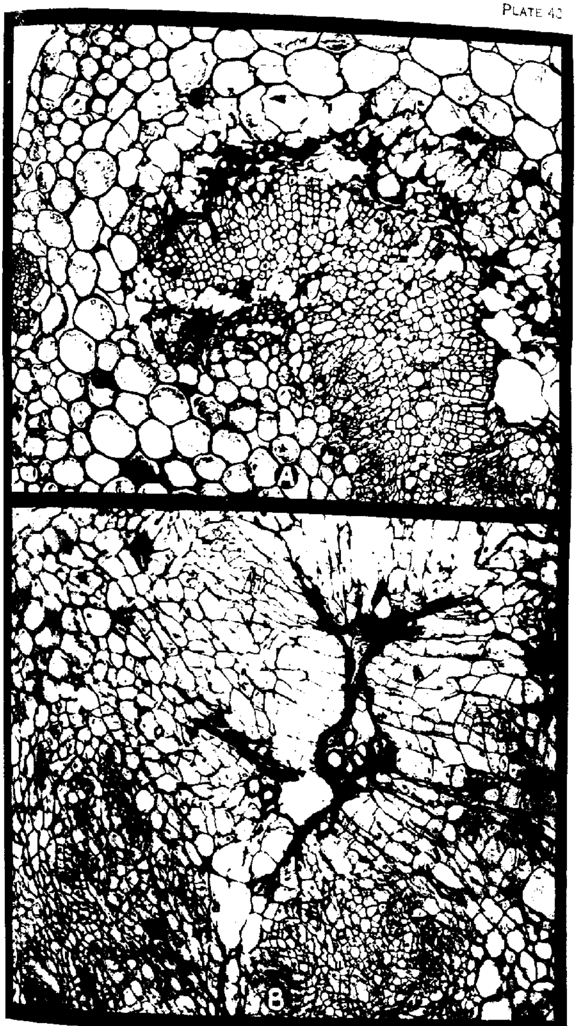
A.—Cross section of internodal region of upper part of stem showing radial stretching of the elements of the pericycle. × 130.

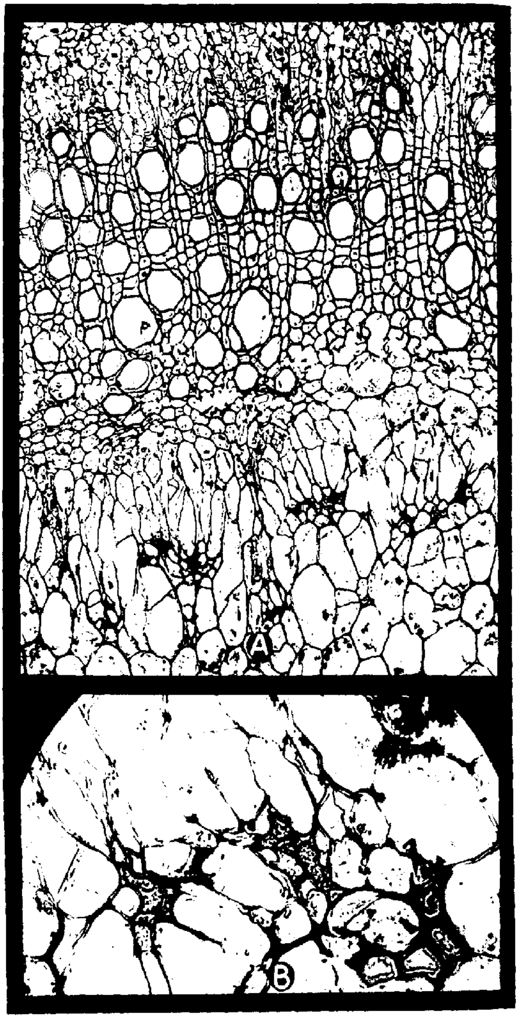
B.—Longitudinal section of nodal region of upper part of stem showing extent of necrosis. × 130.

PLATE 43

A.—Cross section of petiole of mature plant showing severe necrosis. All of the outer phloem and part of the inner phloem groups are destroyed. The adjacent cells of the cortex are also disorganized. × 130.

B.—Cross section of nodal region of stem tip showing formation of large lysigenous cavities extending from cortex to pith and involving inner phloem groups and meta-sylem. × 130.


A
B


A
B

## PLATE 44

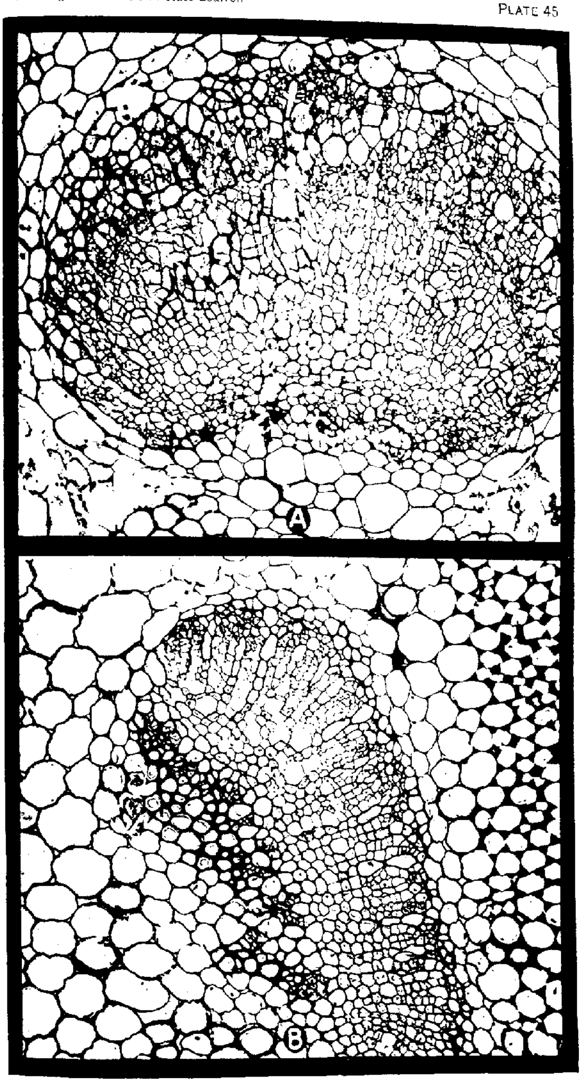
A.—Cross section of stem of mature plant, showing necrosis of internal phloem together with radial elongation of the cells of the perimedullary zone. Outer phloem is well developed and normal. × 130.

B.—Enlarged view of a necrotic internal phloem group. Almost all of the cells in a group are filled with a granular precipitate. × 400.

PLATE 45

A.—Cross section of midrib of mature plants (Magnum Bonum), showing abnormal development of the vascular tissue with thickenings of the walls of the phloem adjacent to the fibers. × 130.

B.—Cross section of petiole of mature plant, showing abnormally large development of the vascular tissue of the petiolar wings. × 130.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